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APPLICATION OF PARTITION CHROMATOGRAPHY TO PROBLEMS OF AMINO ACID METABOLISM*

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The problem of amino acid analysis has attracted much attention in the past, and in recent years numerous attempts have been made to devise techniques suitable for protein analysis. The many difficulties encountered in methods involving specific reagents for the estimation of individual amino acids, have been overcome by using fractionating techniques, of which chromatographic analysis is the most efficient and accurate.

Partition chromatography is the result of Synge's original efforts in trying to resolve the acetyl derivatives of amino acids from wool hydrolysates. He first noticed in 1938 that there was considerable differences in the partition coefficient of many of them between chloroform and water (Synge, 1939). He felt that these differences could be made the basis of a scheme of separation of some amino acids. Using a countercurrent extraction apparatus, with the efficiency of 40 theoretical plates, he found with great disappointment that the technical difficulties were greater than expected (Martin and Synge, 1941). The greatest difficulty in such a continuous system is for the phases to attain equilibrium.

The idea of using continuous extraction as a method of separation was soon discarded in favor of a single process of column filtration. This was accomplished by the ingenious device of immobilizing one liquid in the interstices of a column of an inert gel or fibrous reticulum through which the second solvent is perfused. The solutes appear as separate bands in the column and these bands behave in essentially the same manner as those formed in an adsorption column. For the final estimation, many optical devices have been developed.

Consden, Gordon and Martin (1944) used filter paper as the supporting material for the stationary phase, which is invariably water. The idea in itself was not new. It was an adaptation of the well known capillary analysis on filter paper. The

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principle was new, and the many improvements were very ingenious.

The present report will be concerned only with the use of filter paper chromatography in the study of amino acid metabolism.

Procedure

The solution containing the mixture of amino acids is placed on strips of filter paper in such a way that it forms a small spot about 6 cm. from one end. The strips of paper are placed in a glass trough containing phenol saturated with water. The entire set-up is enclosed in a Pyrex jar which is sealed. The atmosphere in the jar is kept saturated with respect to phenol and water by placing a dish of solvent mixture at the bottom. As the phenol-water travels down the strips of paper by capillarity, the various components of the mixture are carried with it at a rate which is proportional to the partition coefficient of each component. Those amino acids which are more soluble in water move slowest. Those more soluble in phenol move fastest. At the end of a certain period, the strips are dried in an oven, sprayed with ninhydrin in butanol, and, after heating for 10 minutes at 100°, spots representing the various components are revealed. The identity of each spot is recognized by the R_f value, which is the ratio of the distance travelled by the amino acid over the distance travelled by the solvent. The use of one solvent on a strip of paper does not accomplish a complete resolution of all amino acids. Better results are obtained by using a square sheet of filter paper. In a corner is placed the mixture to be analyzed with phenol. After the phenol has travelled a suitable distance, the sheet is removed, dried in an oven at 80° C., and then placed on a second solvent at a 90° angle to the first run. For the second solvent, 2,4-lutidine was found very efficient. Figure I shows a complete resolution of 17 amino acids, using phenol and lutidine.

The possibility of measuring these colored spots quantitatively was not overlooked, and after several attempts we have been able to measure the concentration of amino acids fairly accurately (Awapara, 1949; Landua and Awapara, 1949). For this a chromatogram is prepared. The spots are cut, placed in

tubes, and full color is developed by addition of ninhydrin and heating. An aliquot of the colored solution is removed, made to a proper dilution, and read in the colorimeter.

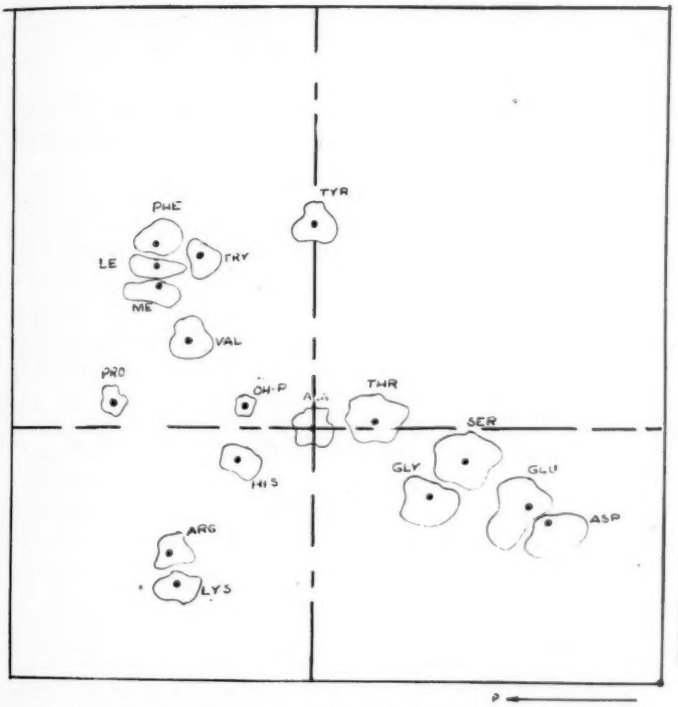

FIG. I. Chromatogram of 17 Amino Acids resolved with Phenol and Lutidine.

The main difficulty in this procedure is the incomplete separation of all the amino acids. Clear and discrete spots are not always obtained. Presently, the quantitative method has been satisfactory for aspartic, glutamic, glycine, and alanine in various tissue extracts (Awapara, 1948).

APPLICATION

Apart from the obvious use of paper chromatography as a means of idetifying amino acids in biological materials, many

other possibilities were considered. In the course of studying amino acid metabolism, it became evident that more specific methods of analysis were needed than those hitherto used. The access to a micromethod, capable of resolving several components made possible the study of amino acid interconversion, since all amino acids could be measured simultaneously on the same sample.

In this laboratory, quantitative paper chromatography was used in the following problems on intermediary metabolism of amino acids.

1. *The Relative Absorption of Intravenously Administered Amino Acids by the Organs of the Rat* (Awapara and Marvin, 1949): The question of how amino acids are concentrated by the organs has been raised many times. Investigations on this subject in the past has thrown some light as to the relative activity of each organ and its capacity to remove amino acids from the circulation. The results reported in the literature (Friedberg and Greenberg, 1947; Luck, 1928; Van Slyke and Meyer, 1913–14) are concerned with total amino acid nitrogen, and no information was gained concerning individual amino acids. The results of the present investigation have shown that not only each organ possesses a specific activity and a typical pattern for concentrating amino acids, but also shows clearly that there are interconversions, such as shown between glutamic and aspartic acid in the kidney (Table I).

2. *Studies on Glycogenesis* (Awapara, Marvin and Wells, 1949): The effect of some adrenocortical principles upon the rate of gluconeogenesis was studied, using adrenalectomized rats with and without adrenal replacement. Amino acids were measured by paper chromatography, and glycogen by standard procedures. The results showed that the dicarboxylic amino acids play an important role in the formation of glycogen from amino acids. In those animals receiving Compound E, significant decrease of dicarboxylic acids in the liver was observed, with a parallel gain in glycogen.

3. *Formation of Taurine and Alanine from Injected Cysteine in the Rat:* The fate of the carbon chain of cysteine in the body has been the subject of much speculation. It has been postulated that it goes into sugar but no explanation for the metab-

olism was given. In this laboratory, it has been shown that injected cysteine in the rat causes an increase in taurine and alanine in the liver as measured by paper chromatography. These findings are in close agreement with Fromageot's scheme, which explains alanine formation in rabbit liver by the action of desulfinicase on cysteine sulfinic acid. The latter compound was shown by Fromegeot to be a very important intermediate in the metabolism of cysteine.

TABLE I

*Concentration of Individual Amino Acids in Organs Fifteen Minutes After Injection of Some Amino Acids**

	Amino Acid Injected	Amino Acids Found per 100 Grams of Fresh Tissue			
		Aspartic Acid	Glutamic Acid	Glycine	Alanine
Liver	Aspartic Acid ..	94 ± 11	87 ± 5	40 ± 7	45 ± 1
	Glutamic Acid..	63 ± 7	120 ± 13	54 ± 9	55 ± 8
	Glycine	60 ± 7	80 ± 11	160 ± 48	53 ± 3
	Alanine	105 ± 14	67 ± 11	45 ± 6	160 ± 16
	None	47 ± 4	60 ± 6	40 ± 6	46 ± 5
Kidney	Aspartic Acid ..	449 ± 31	185 ± 15	62 ± 3	37 ± 2
	Glutamic Acid..	109 ± 5	379 ± 14	66 ± 11	38 ± 5
	Glycine	52 ± 6	99 ± 5	254 ± 7	38 ± 3
	Alanine	43 ± 2	91 ± 8	64 ± 8	167 ± 23
	None	40 ± 3	99 ± 4	63 ± 3	38 ± 5
Muscle	Aspartic Acid ..	55 ± 10	53 ± 7	138 ± 9	44 ± 1
	Glutamic Acid..	56 ± 13	58 ± 4	140 ± 16	47 ± 4
	Glycine	54 ± 17	58 ± 4	190 ± 4	47 ± 3
	Alanine	40 ± 3	40 ± 8	122 ± 14	67 ± 11
	None	38 ± 4	56 ± 2	138 ± 9	38 ± 4

*Arithmetic mean of values from four animals plus or minus the standard errors corrected for small numbers.

4. *Studies on Growth:* As part of an extensive program on growth, the amino acid composition of prostate was studied when that organ was atrophied as the result of castration. The gain in weight of the prostate gland induced by testosterone was followed by parallel analysis of free amino acids. The results indicated that the free amino acids decrease in the atrophied gland and return to normal levels at the end of a 12 day period of testosterone treatment.

Conclusion

Partition chromatography on filter paper is still a new technique which has many limitations. In its present state, it has been of great help in measuring changes in the concentration of amino acids in biological materials. The applications are unlimited in the study of intermediary metabolism of amino acids.

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TUMORS AND MUTATION IN DROSOPHILA*

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Hereditary factors are found to play a role in many and diverse types of neoplastic disease. The use of *Drosophila* as a test animal in the study of genetics in relation to atypical growth presents certain advantages. In this animal, for which genetic data are encyclopedic, tumors are also known. Both hereditary and environmental factors are relatively easily controlled, the chromosome number is small, and the life cycle is short. Numerous tumor stocks have been described and studied in *Drosophila*. Although tumors doubtless appear spontaneously in stocks from time to time, the ones to be mentioned are those which have been especially studied and reported.

Tumor Strains in Drosophila

The first tumor strain reported is the *lethal 7* strain, discovered by Bridges (1916) in one of his cultures when he was studying nondisjunction as a proof of the chromosome theory of heredity. He found one or more black specks in the body cavity of the young larva. These specks proved to be tumors in which there was a deposit of melanin. It was also found that a single gene controlled their production. Stark (1918, 1919b) successfully transplanted these tumors and believed that early death of the larvae was due to the presence of a lethal or malignant tumor. However, further studies by Russell (1940) revealed that because of an abnormality of the midgut in these larvae, they do not retain ingested food after a certain period, and that as originally described, the larvae leave the food, wander onto the side of the vial, and die. She also transplanted these tumors into Canton-S and Oregon larvae with a higher percentage of successes than reported by Stark.

Stark (1919a, 1937) found two mutations which appeared spontaneously in the *lethal 7* strain. A ventral pigmented bar

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appeared on the last abdominal segment, deposited in the hypodermal cells in that location. This could not be separated from the original gene. She also found spontaneous mutation to a benign tumor hereditary in the strain, *be*-3. After obtaining an inbred stock and calculating results from crossover data and outbreeding to marker stocks, it was found (Stark, 1926) that 80% of the effect was due to genes on the second and third chromosome. The gene on the second chromosome was located near the gene, *speck*, on the right end, and that on the third chromosome was located between *roughoid* and *dichaete*. Approximately 20% of the effect was found to be due to genes present on the first and fourth chromosomes. A portion of this work was done by Bridges who also reported (Morgan, Bridges and Sturtevant, 1925) the *benign-b* stock, similar to *benign* 3, which he gave to Stark for analysis. The stock was later lost, however.

Wilson (1924) has described two hereditary tumors in *Drosophila*, *strain* 1 and *strain* 2, obtained from Payne, the first as a strain in which tumors appeared, and the second as a single female with a black spot in the abdomen which was recovered in outcrosses. He concluded that both tumors depend upon multiple factors, some of which are essential and other modifying, the relationships of which are complicated. Some factors caused a greater number of flies to have tumors when homozygous rather than when heterozygous. Both tumors behaved as recessives when crossed to other strains and also when crossed to each other, although there was evidence that they had a factor or factors in common. The action of the tumor factors was modified in some way by genes peculiar to the different strains to which they were crossed. In the first tumor, genes present on the right half of the second chromosome and the right end of the third chromosome were most important. In the second there were main genes on the second and third chromosomes and modifiers on the first and fourth.

Two mutants, probably allelomorphs, were found in two cultures, after X-ray treatment, using a copper and a chromium target respectively, by Gowen (1934). The locus of this gene for *focal melanosis*, *me*, was found to be at 29 on the first chromosome, and it produced a pigmented residue at the lower

tip of the femur or upper end of the tibia. The mode of its inheritance had additional confirmation in a gynandromorph with melanotic lesions on the male half and none on the female half of the fly. It is interesting that another mutation, *ascutex*, located on the chromosome in the region of this gene for *focal melanosis*, causes legs to have blackened, leaky joints, and other abnormalities. It is recognized by Gowen that these characters probably cannot be considered true tumors since the cellular process previous to the deposition of pigment seems to be one of deficiency rather than atypical growth. Gowen, Bridges, and Brehme (1941), also found a new mutant called *melanotic lesions, md*, on the third chromosome producing melanotic lesions throughout the abdomen, thorax, and head. It appeared after benzpyrene had been used. Another mutant which was found after treatment with X-rays was *pigmy (pg)* found by Muller (1935). It caused flies to be dwarfed and melanotic with poor viability, but was lost before further studies could be done. A tumor which was hereditary appeared in a stock of cl^{35k}/Cy in Spencer's laboratory. He found the black tumor to be located within the thorax at the bases of the posterior legs. It was carried as *1(2)1gt* for a time and then lost (Drosophila Inf. Serv., 1938). The viability was poor and flies of both sexes were sterile.

An isogenic stock of Bridges' *tu 36a* strain (Drosophila Inf. Serv., 1938) was bred by Russell (1942), and from it stocks were constructed which were fixed for desired chromosome combinations. (This tumor was found in a *st sr es ro ca* stock originally.) Through a careful study of these, she determined that a factor or group of factors were present on chromosome 2 without which tumors did not appear. In the tumor stock, the X-chromosome increased the incidence of tumors, whereas the third chromosome decreased it. This strain is not to be confused with *tu 36e* discovered by Skoog (Drosophila Inf. Serv., 1938) in an *aa* stock.

Bridges (Morgan, Bridges and Sturtevant, 1925) described a lethal head defect, *1(3)hd*, which killed most of the pupae, presumably as a consequence of black growths in the head. The stock was unanalyzed but was listed by the author in third chromosome stocks. Another tumor affecting the head, *tu-h*,

has been studied by Gardner and was discovered by Stone. This tumor is not melanotic. Gardner and Woolf (Genetics, in press) report a maternal effect controlled by a gene on the X-chromosome in the inheritance of this tumor. Another gene on the third chromosome is required for actual appearance of the tumor. The sex-linked gene is present in *Oregon-R* flies as well.

The stock, *tu^{48}j*, which appeared spontaneously in a *Muller 5* stock of Dr. Herskowitz has been studied in our laboratory. Pigmented spots are found within the body and it is assumed that they represent tumors. The main gene responsible has been found to lie on the second chromosome. Some of the work which has been done on this strain will be discussed later. Other stocks have been described by Morgan and Enzmann and Haskins. Morgan (Drosophila Inf. Serv., 1938) found a melanotic tumor inherited in the brown stock. A detailed analysis of *tu bw* has never been made, but the stock is still in existence. A malformation has been reported in a Notch-8 stock by Enzmann and Haskins (1938). The character appeared as a tumor-like thickening of black color on the dorsum of the fly reaching large proportions on occasion. No microscopic studies were done.

Tumor Genes

The distribution of tumor genes on the chromosomes in *Drosophila* may be summarized in the following manner. The gene responsible for the tumor may be a single gene present on one of the chromosomes, such as the *lethal 7* tumor and *pigmy*. More than one gene may also be active in affecting tumor incidence. They were found present in two chromosomes in the case of the first tumor described by Wilson, on three in *tumor 36a*, and on four chromosomes in the *benign 3* tumor. Not all genes necessarily have the same effect toward tumor production. Some may modify the incidence by increasing it, such as the genes on chromosomes 1 and 4 in the *benign 3* tumor; or others may diminish the incidence, such as the suppressor on chromosome 3 for *tumor 36a*. Tumors which have arisen independently have been found to carry allelomorphs for tumor formation. The data obtained in *Drosophila*

where multiple tumor genes are present are very similar to that for the inheritance of several types of tumors in mice (Burdett, 1948). It should also be noted that in *Drosophila*, as in mammals, the presence of genes for susceptibility to tumors does not always result in 100% incidence.

Morphology of Tumors

No doubt the reason these tumors have been found in *Drosophila* is the presence of pigmentation. Closer study indicates that this is a late change, however. The pigmented bodies, which may be single or multiple, are seen in the adults, pupae, and usually older larvae. When adults are sectioned, only an amorphous mass of pigment, which has the properties of melanin according to Hartung and Tillinghast (1949), is seen. This may also be true in prepupal larvae. Sections made of larvae during the first few days after hatching may show morula-like masses of cells which may reach large size. It is difficult to say whether these cells arise from one locus and later metastasize elsewhere, or whether the tumors arise at several sites independently. It is fairly clear (Stark, 1919 b) that small fragments broken from a primary tumor may thrive elsewhere in the body of the larvae. It is also possible to transplant these tumors to other larvae and have them grow and multiply in the host (Russell, 1940; Stark, 1919b).

Two main types of cells have been described as composing the tumor (Russell, 1940; Stark, 1918). Polygonal cells frequently make up the central mass of the tumor and usually at the periphery are elongated fusiform cells. As the tumor grows older, pigment is seen first at the periphery of the tumor and may be abundant in some regions of the tumor and not in others. Evidently the tumor becomes deeply pigmented and the orginal cells undergo histolysis, leaving a residuum of melanin which persists for the life of the fly. The resolution of the tumor may be related to events in the life cycle. Russell (1940) has pointed out that the behavior of the tumor may be the result of the holometabalous nature of the insect. That is, the tumor regresses at the time of metamorphosis in the same manner as those normal tissues which are replaced by adult

structures. Wilson (1924) postulates some specific substance responsible for the disappearance of the tumor cells.

It is very difficult to say where these tumors arise. The imaginal discs from which adult organs arise have a similar appearance to the cells composing some of the tumors. Sites of origin which have been described are the proventriculus, stomach, a group of phagocytes which Stark calls the blood forming organ, and Weismann's cell chaplet which is the precursor of the adipose body of the adult. In reality, the origin implied has been based on the proximity of tumor cells to the normal tissues named or their similarity microscopically. Stark (1937) and Russell (1940) have published a number of plates depicting the appearance of these tumors.

Effect of Environmental Changes on Tumors

The effects of nutrition on the incidence and growth of tumors has been extensively studied in mammals by Tannenbaum (1947) and others. In general, it has been found that the genesis of tumors in mice may be inhibited by caloric restriction. The growth rate may also be inhibited in a similar manner, but there is also a concomitant loss of body weight. It has been found that the action of genes affecting tumors in *Drosophila* is frequently modified by the environment in which the flies are raised. Wilson pointed out that the percentage of tumors found in the *tumor 1* strain varied inversely with the number of larvae placed upon a given amount of food and also with the number of parent flies used in the mating. The larvae from bottles with less than 20 larvae in them had more and larger tumors in them than when more than 20 larvae were present. Also, the mortality was higher from the effect of the tumor when there was a small number of larvae than when a large number was present. Hartung (1947, 1948) reports that the number of tumors which appear varies with the temperature and that each tumor stock does not necessarily respond to temperature changes in the same manner. The incidence of *tu-h* is increased as the temperature is increased from 15° to 30° C. (12). According to Gardner and Woolf, only the maternal effect is changed by variation in temperature during the first 24 hours of development. Enzmann and Haskins (1938)

and Hartung (1942) have shown that irradiation of tumor strains affects the incidence of tumors. By varying the dosage, Hartung found that there was an increase in tumor incidence with a peak at 1500r, after which the incidence of tumors declined. When the larval stage was prolonged by 2,4-dinitrophenol, Wilson (1947) found that the appearance of tumors in a stock she studied was delayed. A similar effect was noted for high concentration of certain amino acids.

Herskowitz and the author (to be published) have studied the relationship of nutrition to the incidence of pigmented spots in the *tu48j* strain of *Drosophila melanogaster*. The flies were raised in bottles under four types of culture conditions. Stock medium and medium deficient in yeast were used with and without the addition of live yeast on the sixth day. A definite effect of varying the quantity and quality of the food supplies of the larvae was found. Tumors occurred in $29.6 \pm 1.5\%$ of the flies raised on stock reyeasted medium, whereas the same medium not reyeasted yielded an incidence of $19.2 \pm 0.87\%$. An incidence of $14.4 \pm 0.72\%$ was obtained when the flies were raised on poor reyeasted medium and only $0.65 \pm 0.27\%$ of the flies bore tumors when the medium was poor and not reyeasted. The highest percentage of tumors appeared on the second and third day after eclosion, reclining threafter. When the incidence in males was compared with that in females under these different culture conditions, there did not seem to be any differential effect on tumor incidence in the two sexes, although the incidence of tumors in the males present in a single bottle was always lower than the incidence of females in the same bottle. This latter relationship did not necessarily hold when males and females from different bottles were compared. Pupation was delayed by the impaired nutrition, and a plot of the incidence of tumors against a number of individuals counted from each culture suggests an inverse relationship between the two.

Another experiment was performed in the same manner as that described above, except that the number of larvae in the various bottles was kept as constant as possible. It was found that starvation on days two, three and four, resulted in an incidence of $58.9 \pm 3.0\%$ and $60 \pm 2.9\%$ tumors, whereas poor nutrition on days three, four and five, resulted in

$21.4 \pm 2.4\%$ and $15.3 \pm 2.1\%$ tumors. Control values were intermediate between these two extremes. The results of these experiments lend support to the contention that the incidence of tumors is dependent in part on environmental conditions, since the results may not be accounted for by changes in the relative survival of tumorous and non-tumorous individuals. These studies are being continued, controlling the environmental conditions more rigidly, and working with a more uniform tumor stock. Wide variation in incidence of tumors between different cultures in the first experiments may have been due to these two factors.

Mutagenic Activity of Carcinogens

The use of *Drosophila* also affords a different type of approach in the study of the genesis of cancer. One of the more popular hypotheses is that mutations may account at least in part for the change towards susceptibility to cancer. This would lead one to expect carcinogens also to be mutagens. Although it has been known since 1927 that X-rays produce mutations (Muller, 1927), it was not until the work of Auerbach and Robson (1947) that the chemical induction of mutations became firmly established. A most objective way of testing the mutagenic properties of a substance is to determine the lethal mutation rate when *Drosophila melanogaster* is treated with the substance. The mutation rate of *Drosophila* treated with 20-methylcholanthrene was determined (Burdette, to be published) in a series of experiments. Demerec (1948a) has reported positive results using a different method of application of this carcinogen. On the other hand, Auerbach (1939) obtained negative results when 20-methylchololanthrene was tested for mutagenic action.

Females heterozygous for the X-chromosome were treated when three days old by the vaginal douche technique (Herskowitz, 1947) and mated to *Muller-5* males five days old. The virgin females were hybrids from the *Oregon-R* and *Muller-5* stock. F_1 from this cross were tested for lethals. A lethal present on the wild-type chromosome yields males with *sc*^{8} *w*^{a} *B* only, whereas if the lethal is present on the *Muller-5* chromosome, only wild-type males are evident in the F_2.

The treatments were continued serially for a period of 128 days, using eleven generations of flies. These results were compared to those obtained by treating flies with nitrogen mustard and to control, untreated, heterozygous females.

Using the vaginal douche technique, the mortality rate was very high, with and without the carcinogen. In contrast, when the flies were simply treated by dropping the solution on the abdomen, the mortality was diminished by as much as 57%. The fertility was also decreased by the douche technique. It was only 37.2% in the flies surviving treatment with 20-methylcholanthrene in sesame oil. Usually, in the sterile flies no eggs were laid.

A total of 4,660 chromosomes were tested, using 20-methylcholanthrene, and among this number only three lethal mutations occurred, appearing in the third, seventh, and eleventh generations. Two of these were on the *Muller-5* X-chromosome, and one was on the wild-type X-chromosome. Samples from the same solution of carcinogen have been found to produce sarcomas in C_3H mice in approximately one-half the period during which serial administration of the methylcholanthrene to the flies was carried out (Burdette and Strong, 1943). In addition to the lethal mutations, there was a visible mutation to *yellow*, and one case of nondisjunction of the X-chromosome occurred.

In contrast to these results, when nitrogen mustard was applied it was found that 26 lethals appeared in 6,372 chromosomes tested. Fourteen were on the sc^8 w^a *B* chromosome and twelve were on the wild-type X-chromosome.

The percentage of lethals found after treatment with 20-methylcholanthrene was lower than the spontaneous rate for either stock, for the *Oregon-R* stock treated with sesame oil reported by Demerec (1948b), and for untreated heterozygous females similar to those which were treated (Burdette, to be published). Therefore, under the conditions of this experiment there was no increase in the lethal mutation rate induced by the carcinogen. Because of this low number of lethal mutations, and the fact that yellow is known to mutate spontaneously fairly frequently in *Drosophila*, it is unlikely that the visible mutation appeared as a result of the treatment. How-

ever, it should be stated that the experiment was not designed to detect visible mutations or to determine the incidence of nondisjunction.

The following explanations are offered as alternatives for the results. Methylcholanthrene may not be a mutagen, its activity may be strain or species limited, and the route of administration and concentration may not have been proper to bring forth a possible mutagenic action of the chemical. In any event, further work is indicated and is in progress at the present time.

Summary

(1) *Drosophila melanogaster* is a most favorable tumor bearing animal for the study of the genetics of atypical growth.

(2) Certain tumors which have appeared both spontaneously and as the result of treatment have been traced to genes which have been localized specifically on a particular chromosome. These genes may be single or multiple, and in the latter instance the pattern for inheritance of tumor susceptibility is similar to that found in mammals.

(3) The incidence of tumors in susceptible stocks may be modified by the environment.

(4) No support for the idea that 20-methylcholanthrene is a mutagen was obtained, although such a possibility was not entirely excluded.

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SOME ASPECTS OF THE MECHANISM OF ACTION OF IONIZING RADIATION ON LIVING CELLS*

L. G. GRIMMETT

The first experiment on the action of ionizing radiation on living tissues was made some fifty years ago, quite unintentionally, by the French physicist Becquerel, who carried around in his vest pocket some radioactive material which caused ulceration of his chest wall. In the years that followed, the unhappy experiences of the early X-ray workers removed any doubt that radiations of this kind could cause severe damage to living cells.

Since that time, the biological effects of radiation have been widely studied, partly from scientific curiosity, and partly from a desire to understand the processes at work, in order to improve their applications to medicine. Thus, a new science has grown up on the borderlines of physics, chemistry, biology and medicine, which is now known as radiobiology.

At first, the experiments in this field were largely qualitative in character, often consisting of observations of the many different kinds of biological changes which can be produced by radiation. Later on, and especially after the introduction of the roentgen unit as a measure of radiation quantity, the work became much more quantitative in character. Radiobiology can now claim reasonable accuracy on both the biological and physical sides.

The subject is now so vast that I cannot hope, in the short time I have this morning, to do more than touch upon a few aspects of it. I propose to say something about the present views on the fundamental mechanisms of the action of radiation. As many present here today may not be familiar with this field, I shall try to sketch in some of the background as I go along, to show these recent developments in their proper perspective.

By way of introduction to the subject, I would like to show you a short extract from the film on tissue culture growth made

*From The University of Texas, M. D. Anderson Hospital for Cancer Research, Houston. Presented at the 1949 Cancer Conference, M. D. Anderson Hospital for Cancer Research.

in Cambridge, England, by the late Dr. Canti (1928). This classic film was the first in which the technique of time-lapse cinematography was applied to the study of tissue cultures. The method is now quite familiar: the culture is photographed through a microscope at relatively long intervals of time, so that when the film is projected at the usual speed, the life processes of the cells are seen speeded up several hundred times. This particular film shows cultures of chick embryo and of Jensen rat sarcoma. Many cells may be seen to go through the processes of division; and towards the end of the film, the effects of exposing the culture to a mixture of beta- and gamma-rays from radium are shown. It will be noticed that cell division is arrested, proliferation ceases, and finally degeneration of the cell sets in.

Before considering the theories of action of ionizing radiation it might be well to explain exactly what we mean by that term. Ionizing radiations are those whose principal means of dissipation of energy in their passage through matter is the ejection of electrons from the atoms through which they pass. The radiations with which radiobiology is concerned are the α, β, and γ-rays of radioactive substances, X-rays, neutrons and protons. Ionizing radiation can also cause excitation of the atoms through which they pass, but this effect is of secondary importance for our purpose.

All theories of the action of ionizing radiation start from the fundamental assumption of Grotthus and Draper, that it is only absorbed energy which is effective. This is almost axiomatic, for it is difficult to say how the mere passage of radiation unchanged through a cell can produce any effect at all.

There are three principal theories of action: the so-called "point-heat" theory of Dessauer (1932); the target theory of Crowther (1926), Holweck and others (1934); and the more recent chemical theory, elaborated by Fricke (1934), Dale (1943), Weiss (1943), and Lea (1944).

According to the point-heat theory, the ejection of electrons by ionizing radiation is equivalent to the creation of a number of spots of high local temperature in the irradiated medium, giving rise to a kind of microcautery capable of indiscriminate injury to protoplasm in its vicinity. It seems, however, rather

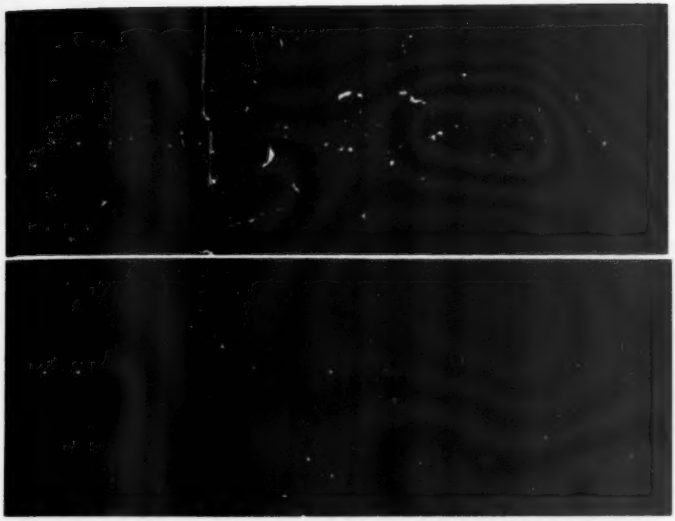
artificial to apply the concept of temperature, which is essentially the property of an assembly of molecules, to a single atom energized by the passage of radiation; and this view has now largely gone out of use.

Before considering the target theory, which has stirred up such lively controversy between physicists and biologists, we might turn aside for a few minutes to consider the purely physical processes which take place when various types of radiation are absorbed. These processes are the same, of course, whether a cell is living or dead, and depend only upon the atoms of which the cell is made.

A great deal of light has been thrown on the problems of absorption of ionizing radiation by means of the beautiful technique of the Wilson cloud chamber. As far back as 1911, Professor C. T. R. Wilson, working in the Cavendish Laboratory at Cambridge, was studying the formation of clouds in air supersaturated with water vapor. In the course of these investigations, he discovered that the tracks of ionizing particles could be made visible by the condensation of tiny water drops on the individual ions. This method has proved extremely powerful in investigations of purely physical phenomena, and also helps very much in consideration of our radiobiological problems. I would like to show you a few examples of the information which cloud chamber photographs can give us.

The series of cloud chamber photographs (Figs. 1–4), show clearly the differences in the nature of the absorption process of radiation of different kinds. X-rays ionize by means of secondary electrons sparsely distributed, protons give denser tracks, and α-rays give tracks so dense that the individual water droplets cannot be distinguished. Linear ion density is of great significance in relation to biological effect, a point to which we shall return later.

So far, we have ony seen pictures of the ionization processes in air and other gases. For biological purposes, of course, we are interested in knowing what happens in the tissues. Nobody has ever succeeded in a direct demonstration of the ionization in water, or in tissues; but there can be no doubt that similar processes take place, although on a much smaller scale,



FIGS. 1–2. Cloud chamber photographs of ionization by secondary electrons from beams of X-rays. Note discontinuous nature of absorption, and sparse distribution of ions along tracks (Crowther).

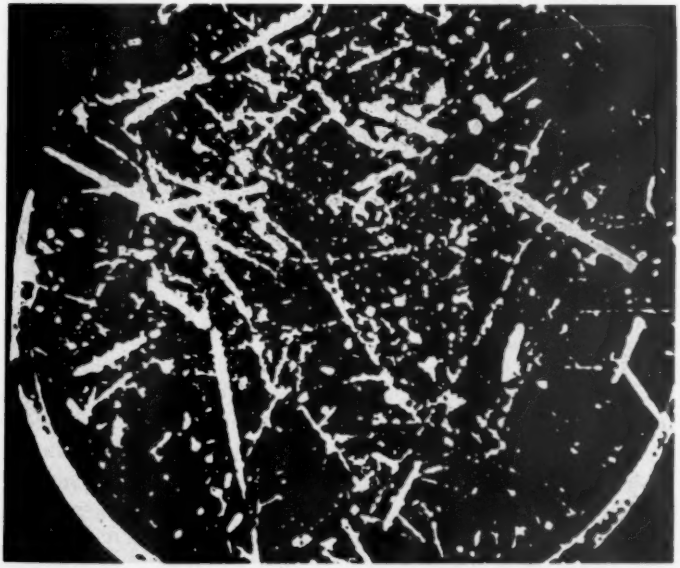


FIG. 3. Cloud chamber photographs of recoil protons produced by neutrons passing through hydrogen. The tracks are denser than those in figures 1 and 2 (Gray).

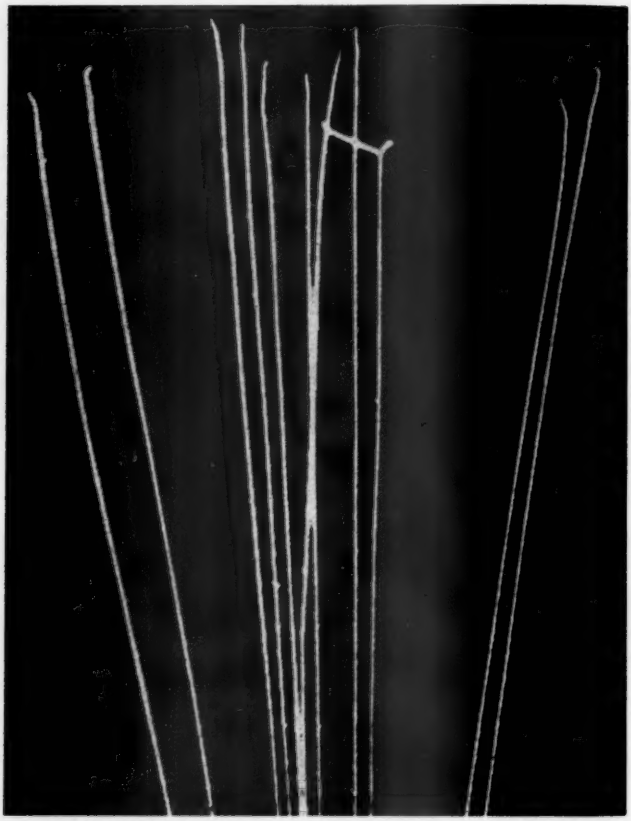


FIG. 4. Cloud chamber photograph of α-rays, showing dense ionization along the track (Rutherford).

approximately in the ratios of the densities of air and water. Fig. 5 shows the kind of ionization pattern we can expect in the tissues for a single 1-million volt electron. Only a few scattered ions are produced; the discontinuity of the absorption process of radiation is very noticeable. In Fig 6 this electron track has been superimposed upon an electron micrograph of bacteria, to the same scale. It will be observed that although the track of this electron actually passes right through a bacterium, it will produce no biological changes, since no ions are

produced in the bacterium itself. The chances of a hit on a smaller object such as bacteriophage are even smaller. It is quite easy to calculate that an object of this size has a good chance of escaping even after administration of a million roentgens!

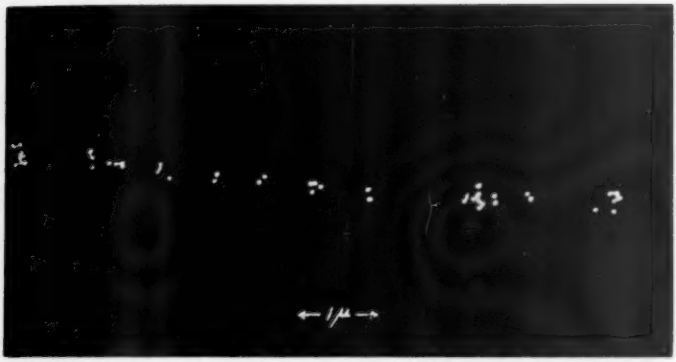


FIG. 5. Scale diagram of ionization produced by a 1 Mev electron in tissues (Gray).

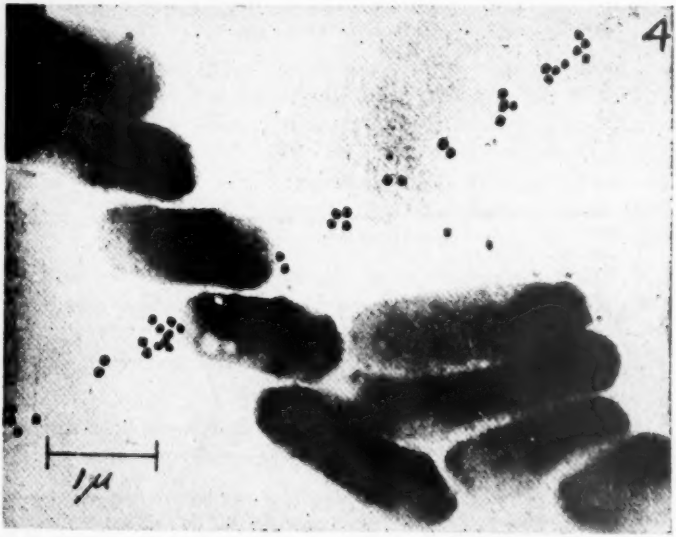


FIG. 6. Electron track of figure 5 superimposed on electron micrograph of *B. coli*, to same scale (Gray).

Keeping these facts in mind, let us return to experimental radiobiology, and consider the types of survival curves which are usually obtained when various organisms are exposed to increasing doses of radiation.

The most common type of survival curve is the sigmoid, but sometimes an exponential curve is obtained (Fig. 7).

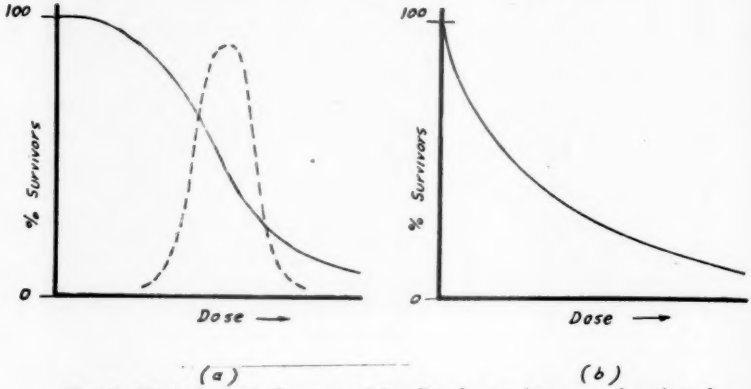


FIG. 7. Typical survival curves of irradiated organisms as a function of dose. (a) Sigmoid curve, differential shown dotted, (b) exponential curve.

The biologists explain these curves by saying that the sensitivities of the organism vary; that some are readily killed by small doses of radiation, while a few resist very large doses.

But Crowther (1926), saw that these survival curves might be nothing more than an expression of the discontinuity of the absorption process, and put forward the so-called "target-theory." He postulated three things:

1) That the sensitivity of all the organisms is the same;
2) That each organism has a small sensitive region, or target, where one, or a few ionic hits kill the organism;
3) That no action takes place until this sensitive spot has received the requisite number of hits.

It can be easily shown that these postulates lead directly to survival curves of the observed type.* For let the volume

*In this example, chosen for its simplicity to illustrate the principle of the target theory, a "hit" is considered equivalent to the absorption of a quantum of radiation in the target. This implies that the radiation is so soft that the secondary electrons are effectively confined to the target. For consideration of other cases see D. E. Lea (9).

of the sensitive zone be v, the absorption coefficient of the radiation be μ, and the number of quanta incident on each sq. cm. of the tissue be χ.

Then the number of quanta absorbed in the target will be:

$$\nu = v \mu \chi$$

If the minimum number of hits on the target required to kill the cell be s, then the probability of a target receiving less than s hits, i.e. the probability of survival, is given by Poisson's equation, well-known to statisticians:

$$P = \left(1 + \frac{\nu}{1^!} + \frac{\nu^2}{2^!} + \frac{\nu^3}{3^!} + \ldots\ldots \frac{\nu(s-1)}{(s-1)^!}\right) e^{-\nu}$$

Fig. 8 shows the family of curves obtained from this expression by giving s the values 1, 2, 3, etc., corresponding to one, two, three or more hits.

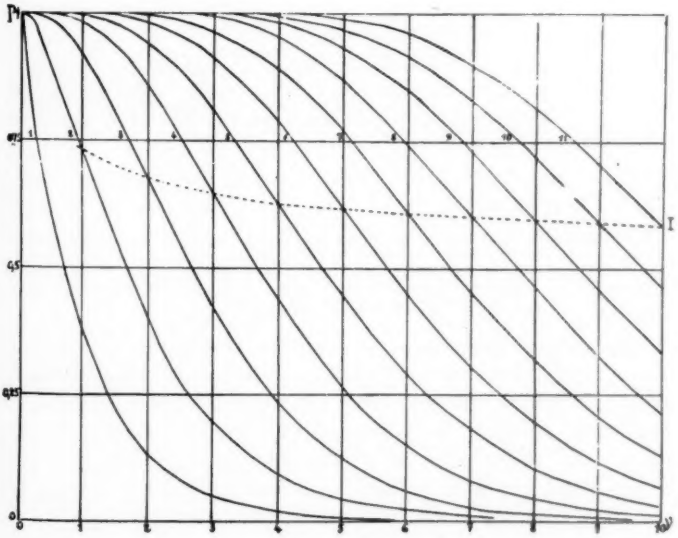


FIG. 8. Family of curves representing Poisson's equation for different values of s indicated on curves (Holweck).

The similarity to the experimental curves is very striking. It should be emphasized that this result does not depend upon any variation of sensitivity from one cell to another, but comes about solely as a consequence of the smallness of the assumed target and the low probability of hitting such a target by means of radiation.

If the theory is true, it gives the immediate possibility of deducing the target from the shape of the survival curve and the known absorption coefficient of the radiation used.

Calculations of this kind have been carried out by Holweck for different sorts of organisms, and he has endeavored to identify the targets with known parts of the organism (Fig. 9).

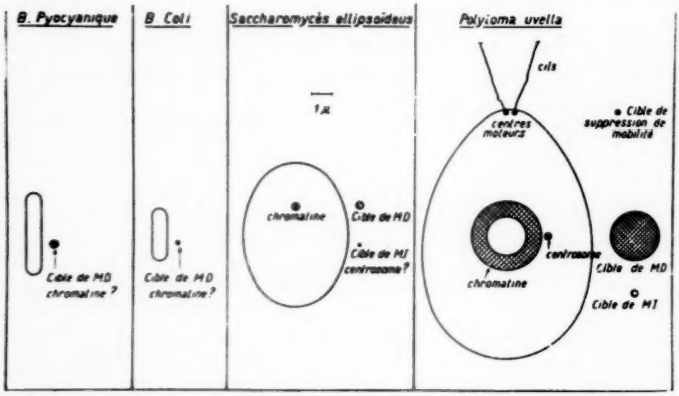


FIG. 9. Comparison of the target sizes for delayed lethal action (mort differée M.D.) and immediate death (mort immediat M.I.), and corresponding components of cell structure (Holweck).

The target theory was not well received by the biologists. They regarded the first postulate, that all of the cells of organisms in a group have the same sensitivity, as quite untenable, and many denied the existence of any specially sensitive spots in a cell. Then again they pointed out that most survival curves were not well defined, and that they could always be matched with one or other of the target theory curves.

There was substance in these objections, and certainly the target theory was used at first in circumstances where its application was doubtful.

It does seem, however, that there is one class of biological effects where the target theory provides the most natural explanation. This is the single-hit type, corresponding to an exponential survival curve. It has been established that in the killing of bacteria by radiation, and the inactivation of certain viruses, the survival curve is strictly exponential to a high degree of accuracy.

If we attempt to account for the shape of this curve by variations of sensitivity among the individual organisms, we run into serious difficulties at once. For it is easy to show that the distribution of sensitivity among a cell population is found by differentiating the survival curve. If we differentiate an exponential curve, we obtain another exponential curve as the expression of the variation of sensitivity amongst the individuals. This would mean that the greater part of the cells have zero expectation of life under a dose of X-rays, however weak. Such an extremely skew distribution of sensitivity seems most improbable.

There are also two further tests which can be made to find out whether a given action is of the single-hit type. One is to vary the intensity of the radiation: if the effect studied is found to depend upon the intensity, in the sense that a larger dose is required to produce the effect at low intensities, then this would be an argument against the one-hit action; for it implies that the action of radiation is cumulative, and that a certain degree of recovery is possible if the radiation is administered slowly enough.

The other test is to try the effect of changing the kind of radiation used. It will be recalled that the linear density of ionization is very different for different radiation. The actual values are shown in the following table.

Now if one hit is sufficient to kill a cell, then a densely ionizing radiation which produced more than one hit in the target volume would be less efficient, for it could still do no more than kill the cell. The extra hits would be superfluous. In other words, the efficiencies of radiations in producing biological effects of the one-hit type should progressively decrease, in the order γ-rays, X-rays, neutrons, α-rays.

Ion Density Due to Various Ionizing Radiations

Radiation	Ionizing Particle	Ions per λ Micron of Tissues
Cosmic Rays	Electron	6.3 (Theoretical minimum for any particle)
20 Mev β-rays (or X-rays)	Electron	8.5
γ-days (Ra)	Electron	11
1 Mv X-rays	Electron	15
200 kv X-rays	Electron	80
Neutrons	Proton	300–1,000
α-radiation (Ra)	α-particle	3,700–4,500
U-Fission	Atomic rays	150,000

Many biological actions have been studied in which all these requirements have been met, and it seems indisputable that the target theory gives the best explanation in this limited field.

On the other hand, there are undoubtedly many experiments which can be explained in terms of variation of sensitivity, and it has been shown recently by Worning that there are some experiments which can be equally well explained by either theory.

So it is to be hoped that physicists and biologists will rest content with this division of the spoils, and agree to lie down once again in peace together!

In conclusion, I would like to say a little on the recent interesting developments in radiochemistry. In the past, the stumbling block in the way of any chemical explanation of the action of radiation was the large dose required to produce recognizable chemical changes, of the order of 10^5 roentgens; as against the 50 roentgens needed to inhibit cell division in tissue cultures, for example.

But now the position has been changed by the experiments of Fricke (1934), and of Dale (1943), and others on very dilute solutions. They have shown that under these conditions the number of molecules brought into reaction is proportional to the number of ions produced in the solution, and is independent of the concentration of the solution. This leads directly to the astonishing conclusion that the action of the radiation is not on the dissolved substance, but on the *solvent*. That is to say water becomes "activated" and capable of reacting with substance dissolved in it.

Two theories of the nature of the activated water have been put forward:

That it is hydrogen peroxide. It has long been known that hydrogen peroxide can be formed from water by the action of radiation; but careful experiments show that very little, if any, hydrogen peroxide can be formed from pure water by irradiation. The presence of dissolved oxygen is necessary for H_2O_2 to appear in quantity.

The more promising explanation is that given by Weiss (1944), that free radicals of hydrogen and hydroxyl are produced under the action of radiations. Absorptions of radiation (OH) ions causes them to lose electrons to the neighboring H^+ ions.

$$H^+ + OH^- + \text{radiation} \longrightarrow HO + H$$

This explanation has the defect that the life of the free radicals would be very short owing to their being liberated close together, with subsequent high rate of recombination.

Lea (1944) has suggested an alternative mechanism:

$$H_2O + \text{radiation} \longrightarrow (H_2O)^+ + \epsilon$$

(The presence of $(H_2O)^+$ has been demonstrated with the mass-spectrograph.)

Subsequently
$$(H_2O)^+ \longrightarrow H^+ + OH$$
$$\epsilon + H_2O \longrightarrow H + OH^-$$

As the electron may wander some distance before reacting, the free radicals will be produced some distance apart, and recombination will be slower than with the first mechanism. These free radicals will have more opportunity to react with molecules of the solute.

If more than one solute is present, there will be competition for the activated water, and there will no longer be a linear relationship between yield and ionization. Dale (1943) has shown, for example, that the radio-sensitivity of enzymes can be diminished by the addition of other organic or inorganic solutes.

Now that it has been demonstrated that significant chemical actions can be produced by relatively small doses of radiation

in the therapeutic range, the possibility of formulating a chemical theory of action is opening up. Experiments with radiation on viruses have shown that the direct action and indirect action via the activated water are both important. Thus it would seem that the new view of radiochemical action can provide a valuable complement to the target theory.

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ANLAGE TUMORS OF THE SALIVARY GLANDS IN UNUSUAL SITES*

BELA HALPERT

Various suggestions have been advanced to explain the structure and cellular origin of anlage tumors of the salivary gland type that formerly were called "mixed tumors of the salivary glands." This older name implies their origin from two of the germ layers, and a close relation to salivary glands. Recently Hellwig (1945) reviewed the principal theories of the cellular origin of these neoplasms and concluded that they are derived from misplaced elements of the notochord. A group of salivary gland tumors segregated by Stewart, Foote and Becker (1945) was designated by them as "mucoepidermoid" tumors. They implicated the salivary gland ducts as the anatomic site of origin. The name "adenomyoepithelioma of the palatal mucous glands" was applied by Bauer and Fox (1945) to another group. These were believed to arise from cells lining the intercalated ducts of the palatine mucous glands.

Our study of the cellular structure of the so-called salivary gland tumors led to the conclusion that they are derived from embryonal rests of the ectoderm. Neoplasms of cell rest or anlage origin reveal themselves by imitating the tissue or organ structure which they originally were destined to form. The pattern of imitation is determined by the cell rests from which they were derived. Depending on the stage of development of the individual at the time when the cells become segregated, these growths may be simple neoplasms mimicking a tissue, or they may be mixed tumors mimicking an organ, or teratomas mimicking an individual (Halpert, 1949).

Adamantinomas are a familiar example of tumors of anlage origin mimicking a tissue. Such growths are composed of ameloblasts, cells which form the enamel of the tooth, the ameloblasts mimicking the structure of a budding enamel body. The adamantinoma or ameloblastoma is an ectodermal epithe-

*From the University of Oklahoma School of Medicine, and the University of Oklahoma Hospitals, Oklahoma City. Presented at the 1949 Cancer Conference, M. D. Anderson Hospital for Cancer Research.

lial growth, the supporting stroma of which is provided by the surrounding connective tissue. Such neoplasms usually occur about the jaw and occasionally at the base of the skull in the region of the sella turcica. Rarely an adamantimoma has been observed also at other locations, like the tibia (Halpert and Dohn, 1947).

The anlage tumors of the salivary gland type are an example of "mixed tumors" mimicking organs. Since these growths do not reproduce or imitate the normal structure of the salivary glands, there is no reason to assume other than an incidental relation between them and the salivary glands. The growths usually occur along the lines of fusion of embryonal facial fissures where salivary glands are numerous, hence the frequent juxtaposition.

The differences in structural patterns between the adamantinomas and the anlage tumors of the salivary gland type may be attributed to at least two factors. One of these is the influence of the environment on the neoplasms in their respective sites. The other is the stage of evolution of the cell rests at the time when they become separated from the ectoderm. Adamantinomas are apparently of more recent genetic origin, hence their simpler range of variation in cellular structure. The anlage tumors of the salivary gland type are genetically older. The stroma in these growths is a part of the neoplasm. The groups of cells from which these neoplasms are derived must have separated from the ectoderm at the stage when they had not as yet acquired their dominant characters as ectodermal and mesodermal cells; hence their ability to differentiate into both ectodermal and mesodermal tissues. In these growths there are marked variations in the cellular patterns.

There are present in varying proportions undifferentiated and differentiated epithelial elements, embryonal connective tissue, adipose tissue, spaces lined by endothelium, young and adult connective tissue, hyaline cartilage, and osseous tissue. There may be observed an apparent transformation of ectodermal elements into mesodermal tissue elements, such as the gradual transition of epithelial cells into cells of hyaline cartilage. The occasional extension of the growth into the nearby glandular tissue without disturbing or distorting

the pattern of the gland appears to be due to juxtaposition rather than to derivation from the salivary gland.

Among the thirty-eight anlage tumors of the salivary gland type observed at the University of Oklahoma Hospitals during fifteen years prior to 1947, twenty-nine were located in or about the parotid glands, six in or about the submandibular glands, and three in the maxilla (Halpert and Tool, 1947). This distribution is similar to that observed by others. These neoplasms also occasionally occur on the head at, or near, embryonal facial fissures at sites where there are usually no salivary glands, such as about the eyes. They have also been observed in locations distant from the head. According to a recent survey, about twenty such neoplasms have been reported in sites other than the head (Halpert and Hackney, in press). Of these, twelve were on the upper extremities (ten on fingers and hands, two on forearms), three on the lower extremities (shin, calf and thigh), and one each in the inguinal region, the vulva, the sternum, and mammary gland.

The group of neoplasms occurring on the head at sites where there are usually no salivary glands, such as about the eyes, and those occurring at sites distant from the head are of special interest. We have observed four such tumors about the eyes and three which were distant from the head; one over the sternum, one in the mammary gland, and one on the back. The tumors over the sternum and in the mammary gland are the second anlage tumors reported in these sites; that on the back is believed to be the first (Halpert and Hackney, in press). The first of these seven patients was seen years ago in the New Haven Hospital (Halpert, 1933); the others were recent patients at the University of Oklahoma Hospitals (Halpert and Hackney, in press). Their histories illustrate the behavior of anlage tumors of this type.

The *first* patient, a 60-year-old man, had a growth of one year's duration on the right side of the bridge of his nose. The removed growth had the shape of a pecan, 3 x 2 cm. Microscopic preparations disclosed, under the thinned skin and subcutaneous tissue, various sized nests of epithelial cells separated by hyalinizing fibrous connective tissue. This in places faded into homogeneous pink stained areas of hyaline cartilage.

In one field there was cartilage transforming into osseous tissue with marrow in the spaces between the bony trabeculae.

The *second* patient, a 49-year-old Negro, had had since childhood a small mass in the center of the left eyebrow. The removed growth was firm, well circumscribed, 3 x 2 cm., and the skin over it was movable. On the cut surfaces there were cavities over 0.5 to 1.5 cm. in diameter containing a brown jelly-like substance. Microscopic preparations disclosed a barely perceptible, delicate capsule separating the growth from the deeper layers of the corium. The growth was composed of sheets of epithelial cells forming no particular pattern and containing occasional spaces or pseudolumina. Some of the large lumina were lined by flat cells and contained an amorphous pink stained material. Occasionally some were lined by stratified squamous epithelium with sheets of keratin in the center. In some fields the cells were arranged in streams. Groups of what seemed to be cells were scattered within an amorphous ground substance resembling cartilage. The connective tissue in some fields was loose and appeared young or embryonal, elsewhere it was more mature and hyalinized.

The *third* patient, a 64-year-old man, had a small, nontender mass that had been present on the right lower eyelid for 40 years. During the past three years it had enlarged progressively, become crusted and caused slight eversion of the eyelid. The excised mass was globular, and 2 x 2.5 cm. in size. The cut surfaces were spongy in appearance, with scattered gray and yellow opaque areas containing minute spaces filled with liquid. Microscopic preparations disclosed nests and strands of epithelial cells arranged in streams or whorls. In places there was a tendency for concentric arrangement with sheets of keratin in the center. Elsewhere, two rows of cell nuclei lined spaces filled with a deep pink stained material. Here and there the cells were within a pink stained ground substance, or in a light stained ground substance resembling cartilage. In the pink stained fields there were some lavender stained granular deposits. There were also groups of adipose tissue cells.

The *fourth* patient, a 52-year-old man, complained of a mass in the left lower eyelid that had been present for about three

years. Microscopic preparation from a biopsy specimen disclosed sheets and nests of epithelial cells within a connective tissue stroma. The latter in places was composed of a fibrillar ground substance containing occasional nuclei, the cytoplasm of which faded into the ground substance. Strands of epithelial cells formed a lacelike pattern within this ground substance adjacent to the larger cell nests.

The *fifth* patient, a 79-year-old man, had a non-tender mass in the right mammary gland that had been present for the past 40 years. It had enlarged slowly, had never been painful, and no discharge from the nipple was ever noted. The amputated right mammary gland contained immediately below the nipple a globular, slightly lobulated, well circumscribed, firm mass, 3 x 2 cm. in size. On the cut surface the mass bulged somewhat, was yellow-gray, and had a lobular pattern with occasional areas of red-brown discoloration. Beneath the nipple and near the adjacent margin of the growth there was a gray-white area 0.7 cm. in diameter which gave a grating sensation when cut. In microscopic preparations, the periphery of the growth was delimited by a hyalinizing fibrous connective tissue capsule. Sheets and nests of epithelial cells were seen within a loose connective tissue stroma. Scattered through many fields were lavender stained concentric bodies. Elsewhere, lumina were lined by cubidal cells. These were within a pink stained, dense or loose, and fibrillar ground substance. Other spaces were lined by columnar cells, or the nuclei of the cells were in several rows. Over extensive areas the cellular pattern was blotted out by hyaline change. There were no areas of cartilaginous or osseous tissue.

The *sixth* patient, an 84-year-old man, stated that six years previously a small "cyst" over the lower sternum had been cauterized, but the lesion persisted and periodically drained a thick white material. On the cut surfaces of the excised lesion there was a gray-white, firm mass, 1.2 x 0.9 cm., which consisted of lobules up to 0.5 cm. in diameter. Microscopic preparations disclosed sheets of neoplastic cells in a loose or more dense hyalinizing fibrous connective tissue stroma. Here and there the epithelial cells formed acinar or tubular structures lined by flat, cuboidal or columnar cells.

Some of the nests were composed of cells with round or oval nuclei in a halo-like cytoplasm. These were in streams or whorls in a scanty stroma. Elsewhere strands and nests of epithelial cells formed no particular structures and appeared within a pink stained ground substance. Again, other nests of cells had a pink stained cytoplasm and eccentrically placed nuclei not unlike the acidophilic cells in the hypophysis. In still other fields, lumina or pseudolumina were filled with a pink stained vacuolated or scalloped coagulum of varying shades.

The *seventh* patient, a 79-year-old man, said that about four years previously a small mass appeared in the right upper lumbar region which enlarged progressively, but caused no discomfort until one year ago when it became tender to touch. The specimen removed consisted of an elliptic portion of tissue, 11 x 7 x 5.5 cm. in size, covered with skin. The opposite surface was raw, with lobules of adipose tissue attached. On the cut surfaces there were several cavities up to 3 cm. in diameter containing dark red fluid. Bordering these cavaties was a soft and firm, gray-yellow tisse. In microscopic preparations there were strands and nests of what appeared to be epithelial cells within a scanty, loose, partly hyalinized connective tissue stroma containing blood vessels. Epithelial cells enclosed various sized, slitlike, round or oval spaces. Extensive areas of necrosis were discernible. An apparent condensation of fibrous connective tissue formed a capsule at the periphery. In other preparations, strands of epithelial cells were seen in a fibrillar ground substance resembling mucin, or in a pink stained ground substance resembling cartilage. The epithelial-like cells faded into cartilage cells.

Comment

On close scrutiny of the microscopic structure, no fundamental difference is disclosed between the anlage tumors of the salivary gland type occurring in sites usual for these growths and those occurring elsewhere. In the latter there tends to be a less complicated pattern with a predominance of the epithelial elements. There is also less tendency to the production of cartilage and other mesodermal elements. These observations, as a whole, reinforce the concept of the anlage

origin of these growths. They also support the idea that the proximity to salivary glands is incidental rather than essential. The variations in cellular patterns may be attributed to local influences on the anlage from which the growths are derived.

Cells left over in the process of evolution of the individual are present at many sites. These cells usually remain dormant. If they commence to proliferate they may reproduce a complete pattern forming aberrant organs, or they may develop into so-called benign neoplasms, or they may form cancerous growths. The factors initiating and governing the behavior of these cell rests, as well as the fundamental problems of normal and atypical growth, still remain subject for future investigations.

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ENDOCRINE AND VITAMIN FACTORS IN HORMONE-INDUCED TISSUE GROWTH*

ROY HERTZ

This paper is concerned with the problem of the interaction of the vitamins and hormones in the experimental induction of tissue growth—more particularly with the type of tissue growth which can be induced in the female genital tract by the administration of estrogens. We want to discuss the interaction between biotin and folic acid, two recently identified members of the B complex, in relationship to that type of hormone-induced tissue growth.

Now, first, with respect to biotin. Biotin was first recognized as a growth essential for certain strains of yeast. Subsequently, through nutritional studies, it became apparent that biotin is the nutritional factor which protects against egg white injury in the rat. Raw egg white contains a factor which is termed "avidin." Avidin has an affinity for the biotin content of the diet and forms an unabsorbable biotin-avidin complex. In that way the animal is indirectly made biotin deficient. The condition results in an extensive scaly dermatitis. There is also marked alopecia, spasticity of the hind limbs, marked diarrhea and in the latter part of the course of the animal's illness there develops a panmyelophthisis with a hemorrhagic tendency, with death soon occurring. Now this entire effect of the feeding of raw egg white can be prevented by the administration of as little as two-tenths of a microgram of biotin per rat per day.

This entire problem was approached from the endocrine point of view, since the albumin of the hen's egg is a mucoid secretion from a specific part of the genital tract of the hen. The yolk of the egg is formed in the ovary and the yolk is then picked up in the gut-like oviduct passing by peristalsis down the genital tract. As it descends the tract, the albumin is secreted in concentric layers about the yolk, then the shell is

*From the National Cancer Institute, Bethesda, Maryland. Presented at the 1949 Cancer Conference, M. D. Anderson Hospital for Cancer Research.

formed and the egg passes on to the outside. It was thought that this secretory portion of the oviduct must be the source of the anti-biotin factor. Employing homogenates of this portion of the genital tract and a yeast growth test which shows a specefic biotin-reversible inhibition, it was demonstrated that this tissue did in fact contain large amounts of the growth inhibitory substance.

Furthermore, it was found that the oviduct of the non-laying hen, that is the hen which had stopped laying, contained in its genital tract no anti-biotin. This meant the ovarian activity and presumably steroid secretion was essential for the induction of this anti-biotin formation.

Next, sexually immature chicks were subjected to experiments designed to induce anti-biotin secretions in their genital tracts. Large doses of estrogen administered to these chicks induced a 40-50 fold increment in tissue mass over a period of six days. However, the tissue was found to contain no anti-biotin. If both estrogen and progesterone were administered together this mucous membrane became transformed into active secretory tissue (comparable to the premenstrual endometrium of the primate or the pseudopregnant endometrium of the rabbit); and a large amount of anti-biotin could be readily demonstrated. (Hertz, 1946) (Hertz, Fraps and Sebrell, 1943) (Fraps, Hertz, Sebrell, 1943).

Now to make matters somewhat more complex, Trager (1947), has shown that there is a fat soluble substance in the serum of the horse and in the serum of many mammalian species which, although not biotin, can replace biotin as a growth essential for several strains of bacteria. The administration of estrogens to a chick produces a marked lipaemia, the serum lipids sometimes rising to levels 400–500% above normal. Also with the administration of either estrogen alone or estrogen plus progesterone, there is a marked increase in the overall biotin activity in the serum. This increase is referable either to the fat soluble factor or to the chemically identifiable biotin factor in the same proportions (Hertz, Dhyse, and Tullner; in press). Therefore it would appear that the anti-biotin substance, avidin, remains within the tissue of the genital tract

and does not circulate. This is probably explained by the fact that avidin is composed of a rather large basic protein molecule.

The problem of the relationship of folic acid to estrogen-induced tissue growth is somewhat different. Before folic acid was chemically identified, it was found that certain synthetic diets, minus folic acid and several, as yet, unidentified factors of the B complex, fed to chicks and to monkeys failed to support optimum estrogen induced tissue growth. When animals were on these synthetic diets, instead of inducing a 40 fold increment in tissue growth in the genital tract, the folic acid deficient chick showed only a 2–3 fold increment in tissue mass even when massive doses of estrogens were administered (Hertz, 1945). In addition riboflavin deficient chicks or pantothenic acid deficient chicks gave nearly optimal responses to high doses of estrogen, and this suggested some degree of specificity in the relationship of folic acid to the utilization of estrogens.

More recently, there have become available through the application of the principal of competitive molecular analogues, a number of antagonists to folic acid. Aminopterin or 4-aminopteroylglutamic acid is one of them. Since estrogen-induced tissue growth depends on optimum folic acid intake, this antifolic acid substance quantitatively inhibits tissue growth response in the genital tract of both the chick and the rat (Hertz, Tullner, 1949). The normal oviduct weight in a three-day-old chick is about 10 mg. In 48 hours under estrogen administration this may be increased to about 50 mgns. Now, if to a fixed massive dose of estrogen increasing quantities of folic acid antagonists are added, there is a progressive decrement in tissue growth response. However, if to an effective inhibitory dose of antifolic an excessive amount of folic acid is added, there is a reversal of this inhibition and the tissue growth response returns almost completely to the expected level. When chicks remain first for two weeks on a folic acid deficient diet so that they are depleted of folic acid, the oviduct weighs about 25 mg.; whereas folic acid deficient estrogen-treated animals show oviducts weighing about 150 mg. Now if to this folic acid, deficient diet during six days of estrogen administration, one hundred micrograms of folic acid per chick per day are added, weight of the

oviduct rises to 850 mg. If this dose of folic acid is kept constant at 100 micrograms per chick per day and progressively larger amounts of anti-folic acid are added to it, there results progressive decrements in response. Finally 50 micrograms of the folic antagonist, aminopterin, will completely neutralize the growth stimulating effect of 100 micrograms of folic acid. This then gives a titration for tissue growth response, since one part of aminopterin inhibits two parts of folic acid. This inhibitory ratio is just about what has been found for several strains of bacteria requiring folic acid. It would seem that this is similar (if not the same) type of growth phenomenon which is found in bacterial cultures.

Similar observations were made with the rat uterus. The uterus of the sexually immature rat weighs about 25 mg. Estradiol, in 48 hours, will increase the uterine weight to 80 mg. As increasing doses of anti-folic are added, there is a progressive decrement in the growth response. The addition of excessive folic acid results in a substantial reversal of the inhibition quite parallel with that seen in the chick.

Thymine (5 methyl-uracil) has been observed to replace folic acid for certain strains of bacteria as a growth essential. It is also effective in cases of macrocytic anemia which are also cured by the administration of folic acid. This phenomenon has led to the belief that folic acid functions in some way in the incorporation of purines and pyrimidines into nucleic acids.

Consequently it was thought that it would be of interest to see what some of the anti-purine and anti-pyrimidine compounds would do. One of his compounds, 2-6 diaminopurine, (which is the direct analogue of adenine), was found in fact to be a very potent inhibitor of estrogen induced tissue growth (Hertz and Tullner, 1949, b). Now if to this dose of diaminopurine is added an excess of adenine, a substantial reversal of the inhibition occurs.

In summary: 1. The production of a specific growth inhibitory substance, namely avidin, in the female genital tract appears to be under specific steroid hormonal control and subject to quantitative experimental study. 2. Hormone-induced tissue growth is vulnerable to inhibitors of nutrilites which

appear to be quite essential for this type of tissue-growth response.

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ENZYMATIC PATTERNS IN TUMOR METABOLISM*

G. A. LePage

The living cell probably has, at a conservative estimate, several hundred chemical transformations to accomplish in carrying out its metabolism. It does not leave these chemical transformations to chance. It must regulate them and promote them at the proper rates. Therefore, for every chemical transformation that the cell carries out in its metabolism, it appears to have one or more catalysts—organic catalysts which we refer to as enzymes. The living cell, then, in the view of the enzymologist, is a family of enzymes. These are kept in balance by a variety of secondary factors such as the hormones, and one very interesting approach to the study of cancer is the study of these enzymes in terms of their patterns in normal and in tumor cells. The first detailed study that was made of the enzyme patterns in tumor tissue was made over twenty years ago by Warburg (1926), who said: "Interference with the respiration in growing cells is, from the standpoint of the physiology of metabolism, the cause of tumours. If the respiration of a growing cell is disturbed, as a rule the cell dies. If it does not die, a tumour cell results. This is no theory, but a comprehensive summary of all the measurements at present available." As far as the research in enzymes has indicated in the ensuing years, this statement is probably still correct.

The enzyme approach to the cancer problem is best exemplified by the following four questions. 1. What is the enzyme pattern that characterizes cancer tissue? 2. How is the enzyme pattern of normal tissue converted to the cancer pattern? 3. How are enzymes of normal tissue organized so as to prevent unlimited growth? 4. How can we block strategic enzymes in cancer tissue without damaging normal tissue? I do not have the answers to these four questions. If I did we would probably be a great deal closer to the answers to the cancer problem. But

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I propose to give you some examples of the type of project we are carrying out in enzyme research in this matter.

Figure 1 is a chart that was prepared several years ago by Potter (1943), in our laboratory at the University of Wis-

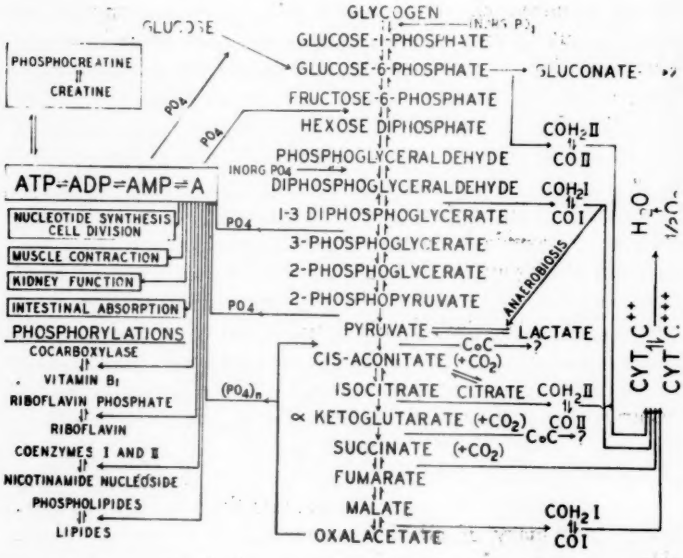

FIG. 1. A chart of the reactions of carbohydrate metabolism, arrows representing enzyme-catalyzed reactions.

consin. It illustrates most if not all the information we have on the metabolism of carbohydrates in the cell in terms of the enzymatic reactions. All the arrows indicate enzymatically catalyzed reactions. This appears somewhat complicated, but it is actually a masterpiece of design when one considers how many reactions are illustrated and that none of them is confused by the crossing of any of the arrows. In the metabolism of glucose, which is probably our most generally available substrate in tissues, glucose is first phosphorylated to a compound known as glucose-6-phosphate, and that reaction can be backed up to glycogen when carbohydrates are being stored. But when the glucose is being utilized directly it is carried through the

series of reactions illustrated in Figure 3, which result in the production of lactic acid or pyruvic acid. In the metabolism of the normal tissues, the pyruvate resulting from this series of reactions in the metabolism of glucose is condensed with a compound known as oxalacetic acid to yield the compound known as cis-aconitic acid, which is carried through a cyclic-reaction resulting in regeneration of the oxalacetic acid. The net result, then, is the oxidation of the pyruvic acid to carbon dioxide and water and the regeneration of the oxalacetic acid. Within the bounds of this cycle it is possible to show where fats fit into the series of reactions. Fats are broken down to acetate and aceto-acetate, and these compounds feed into the cycle of carboxylic acids. Likewise, the proteins fit into that cycle and are metabolized first by their deamination.

For example, glutonic acid, when it is deaminated, is converted to ∝ -ketoglutaric acid which is a member of this carboxylic acidcycle. When Warburg examined the metabolic pattern in tumor tissue by the use of tissue slices, he found that tumor tissue was characterized by being able to convert glucose to lactic acid at a relatively rapid rate. He found that if one measured the glucose and lactic acid in the blood entering the tumor and in the venous blood leaving the tumor, there was considerable change. If the glucose was 100 mg. per cent on entering the tumor, it would be in the neighborhood of 45 mg. per cent when it came out in the venous blood from the tumor. A corresponding amount of lactic acid appeared in this venous blood so that he was able to calculate that a large part of the tumor's energy was derived from this series of reactions, the conversion of glucose to lactic acid. In contrast to the process in normal tissue, where it was completely oxidized to carbon dioide and water the lactic acid produced in tumor tissue was put into the blood as such. Now, as a result of these studies, the impression seems to have grown up in the literature that tumor tissue is a very rapidly glycolyzing tissue and that other tissues do not glycolyze so rapidly. This, of course, is not true.

In our studies of glycolysis, comparing normal tissues of the rat with rat tumors, we used preparations of cells which were first broken up in a glass grinding machine which we speak of

as a homogenizer, so that we were dealing not with intact cells, but with cellular contents which we could dilute and to which we could add back any necessary factors to obtain the reactions listed in this chart. When we did that we were able to get away from certain limitations, the limitations of permeability factors which are present in work with tissue slices. For example, when one works with tissue slices, one cannot use the phosphorylated intermediates which have been correlated, because the tissue cells, unless they have been quite damaged, are not permeable to these compounds. We find that when we add glucose and the phosphorylated intermediates, hexose-diphosphate, fructose-6-phosphate, or glucose-6-phosphate to homogenates of rat tissues then we can obtain lactic acid production—the energy of these reactions being converted to phosphate bond energy which maintains the reaction rates. In our reaction mixture we added a relatively small amount of hexose-diphosphate and a relatively large amount of glucose. We wanted to find out if the phophorylation by which glucose is converted to hexose-diphosphate was a limiting reaction in glycolysis of these various tissues. We found, when each of the rat tissues was studied in this manner, that brain and tumor homogenates were able to convert the hexose-diphosphate to lactic acid rapidly. They were also able to phosphorylate some of the glucose and produce more of the hexose-diphosphate so that the reaction continued. In contrast, liver, kidney, and diaphragm muscle, taken as examples of other normal tissues, were able to metabolize the hexose-diphophate to lactic acid, but they were not able to phosphorylate glucose and produce a further supply of hexose-diphosphate. This is illustrated in Figure 2.

In the next series of experiments, we added hexose-diphosphate in excess to see what the potential rate of reaction was with all the glycolytic enzymes below this phosphorylation step. We found that in a typical rat tumor, the Flexner-Jobling carcinoma, the rate of lactic acid production, shown in Figure 3 in units which represent the amount of carbon dioxide produced from bicarbonate buffer medium per hour per milligram dry weight of tissue, was approximately 50. Brain was

also approximately 50, and the other tissues were considerably higher in activity. That is, with the exception of this phosphorylation step, the normal tissues are actually able to glycolyze more rapidly than is the tumor. Therefore, in looking for a difference between the tumor and normal tissues, we must look to some other part of the metabolism.

Glycolysis in rat tissue homogenates with glucose + 6 micromoles HDP added

Tissue	Lactic Acid Prod. micromoles	P esterified micromoles
Brain	10	6.8
Tumor	10	4.5
Liver	6.1	0
Kidney	6.0	0
Diaphragm	5.9	0

FIG. 2. Glycolysis by homogenates of rat tissues with a 3.0 ml. reaction medium in Warburg respirometer flasks, using 30 mg. of tissue and a 40 minute incubation at 37.5°C. Conditions were anaerobic (95 per cent N_2 — 5 per cent CO_2).

Glycolysis in rat tissue homogenates with excess HDP

Tissue	Qlactic
Flexner-Jobling rat carcinoma	50
Brain	50
Liver	90
Kidney	110
Diaphragm	150

FIG. 3. As in the previous figure with an excess of hexosediphosphate (HDP).

A study has been made by Potter in our laboratory of the enzymes concerned in the steps of metabolism between lactic

acid and carbon dioxide and water, the final products of carbohydrate metabolism. Certain of the enzymes of that system were considerably deficient in tumor, as compared with any of the normal tissues. However, a more important finding has come to light rather recently—one of these enzymes of the aerobic carboxylic acid cycle was not just low in tumor tissue relative to the normal tissue, but appeared to be completely absent from tumor tissue (Fig 6). This is the enzyme which oxidizes oxalacetic acid. One can see that all the normal tissues listed in Figure 4 are able to oxidize oxalacetic acid at a very

Rate of oxidation of oxalacetic acid in rat tissue homogenates

Tissue	Q_{O_2}
Kidney	52
Liver	16
Heart	26
Brain	15
Flexner-Jobling carcinoma	0

FIG. 4. Oxidation of oxalacetic acid by homogenates of rat tissues supplemented with all necessary cofactors. Oxygen uptakes are expressed as cu. mm. of gas taken up per mg. dry weight of tissue per hour.

appreciable rate, whereas Flexner-Jobling carcinoma appears to have none of this enzyme. The true importance of this has come to light still more recently from the work of Mitchell and Houlahan (1947). In their work with a mold, known as *Neurospora crassa*, they made use of X-ray mutants. They were able to demonstrate that this compound, oxalacetic acid, was a building block in the synthesis of uracil. Uracil is a building block for nucleic acid. Of course, the main difference we observe metabolically between cancer tissue and normal tissue is that cancer tissue continues to grow in an uncontrolled manner, whereas normal tissues stop multiplying upon reaching maturity. Some control mechanism is brought to bear to stop growth and this control mechanism is apparently missing

in the tumor cells. One possible explanation for this would be that the normal tissues reach a point where they are deprived of some key building block for growth. In growth we must necessarily have the synthesis of nucleic acid. This is one possibility where tissues might impose a limitation of building blocks. That oxalacetic acid oxidase is missing from tumor tissue may make it possible for oxalacetic acid to be more available for the synthesis of uracil and thereby permit the formation of nucleic acids and the continuation of growth. This concept has been strengthened by recent work in Sweden in which an intermediate further along the chain of reactions was utilized in the synthesis of uracil. These Swedish workers, Arvidson et al. (1949), used a compound known as orotic acid which is an intermediate in this chain of reactions found by Mitchell and his co-workers. They injected orotic acid which had been labeled with Nitrogen 15 into rats and found a very rapid incorporation of this compound into the uracil of the rat tissues. Figure 5 illustrates some of the reactions we know that go on

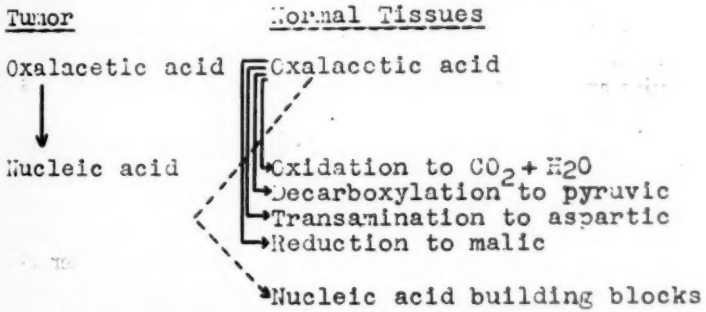

FIG. 5. Illustration of some reactions involving oxalacetic acid in the cell.

in normal tissues, by which oxalacetic acid is transformed. The tissues can oxidize it to carbon dioxide and water. The mechanism for this is a very powerful system, at times operating at almost infinitesimal levels of oxalacetic acid. They can decarboxylate it to pyruvic acid. They can transaminate it to aspartic acid, or they can reduce it to malic acid, which is in the carboxylic cycle. If the normal tissue is to grow, it presumably must use this oxalacetic acid as a building block for

growth. The reaction by which it makes nucleic acid from oxalacetic acid has to get by all competing reactions. In the tumor the oxidation to carbon dioxide and water, and decarboxylation to pyruvic acid are essentially missing, so that any process using oxalacetic acid to synthesize nucleic acid would be freed from those competitors. In examining other rat tissues, Potter has found that thymus also was low in the oxalacetic acid oxidase enzyme.

Studies have been reported in the literature in which radioactive phosphorous was used to determine which tissues of the body were actually growing. The data has been discussed by Hevesy (1947). This was done by isolating the desoxyribonucleic acid from the nuclei of the normal tissues after the injection of radioactive phosphorus and determining the activity there. The workers who did this found that thymus was a very rapidly growing tissue and this finding is consistent with the other data regarding the ability to grow when oxalacetic acid is made available as a building block. Figure 6 is an illustration of the reactions that Mitchell and Houlahan (1947), found to be involved in the synthesis of uracil using oxalacetic acid as a building block. Oxalacetic acid is aminated to amino-glutaric-amide and this is carboxylated to orotic acid which in turn is converted to uracil.

We realize that there are any number of other possibilities for limiting a building block in tumor or normal tissue and thereby affecting the growth rates; but this is one promising lead, and it is one which is amenable to experimental procedure. Such experiments are now in progress. It is interesting to not that Brown et al. (1948), found, using Nitrogen 15 as a tracer, that uracil injected into the animal as such was not utilized for the formation of nucleic acid. In other words, the cell has to make its own uracil. In contrast, the animal is apparently able to utilize injected adenine and is able to make guanine from adenine. This strengthens the concept that uracil might be a limiting building block for growth.

Dr. Ira Telford: I would like to ask whether you studied the regenerating liver tissue for oxalacetic acid oxidase?

Dr. LePage: Regenerating liver has not been tested.

Dr. W. A. Selle: What would iodoacetic acid do to this reaction?

Dr. LePage: Iodoacetic acid is known to be an inhibitor for the reaction by which triosephosphate is oxidized to phosphoglyceric acid, and would hence stop glycolysis before it yields any phophate bond energy.

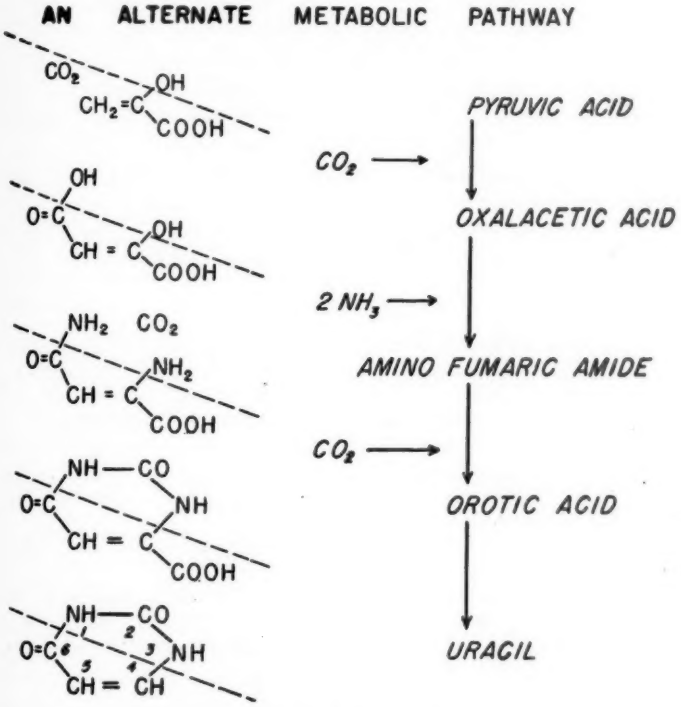

FIG. 6. An illustration of the manner in which oxalacetic acid can be used as a building block for uracil synthesis.

Dr. Ira Telford: I would like to ask if oxalacetic acid oxidase is a single enzyme or several enzymes?

Dr. LePage. What we refer to as oxalacetic oxidase is actually a system of enzymes by which oxalacetic acid is carried around the carboxylic acid cycle.

Dr. Ira Telford: In other words, where you measured oxalacetic oxidase you were not measuring a one-step oxidation?

Dr. LePage: No. The results are expressed here on the basis of oxygen uptake with oxalacetic acid as substrate, but the results could also be expressed as disappearance of oxalacetic acid from measurements of keto acid. Succinoxidase assays are lower in tumors than they are in normal tissue except that skin is lower in succinoxidase than are skin tumors. The succinoxidase and malic dehydrogenase enzymes are present in tumor tissue with a difference only in the quantity. We have shown that cancer tissue is able to form oxalacetic acid from succinic or malic acids. Whether the tumor makes oxalacetic from these, obtaining them from the blood or whether it makes oxalacetic acid from pyruvic acid and carbon dioxide, we do not know. We are making studies now with radioactive carbon to determine this answer.

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ELECTROPHORETIC STUDIES OF THE PLASMA PROTEIN CHANGES IN NEOPLASTIC DISEASES*

JOHN W. MEHL

In a general way, it is quite obvious that the study of blood has been of considerable value in the past in helping to understand processes taking place in tissue. Abnormalities in the metabolism of tissues are often reflected in the composition of the blood, and when a particular component of the blood is specifically regulated by a particular tissue, the manifestations in the blood may be quite useful in evaluating the function of the tissue. In other cases, the changes in the blood may represent secondary effects on the body economy due to metabolic abnormalities in a particular tissue, but the resulting changes in blood may be non-specific with respect to the tissue which is primarily involved. It is, of course, in the hope that some specific and characteristic change may be found, or in the hope that the changes will contribute to a better understanding of the pathological process, that the plasma proteins have been investigated in almost all diseases. To a considerable extent, such studies have been carried out with no clear idea of what plasma protein fraction might be involved, since our knowledge of the source and function of the plasma proteins has often been rather vague. Early studies were largely limited to rather crude separations into albumin and globulin fractions and fibrinogen. The development of the electrophoretic method of analysis by Tiselius (1937), represented a very great advance in that it made possible the study of changes in the albumin and at least four globulin fractions other than fibrinogen by an objective and reproducible method. However, even this now seems relatively crude, for the plasma fraction studies of Cohn and co-workers (1946), have served to call particular attention to the fact that the electrophoretic globulin components are gross mixtures. Indeed, if we recall what is known of the multiplicity of functions which must be assigned to the

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plasma proteins it seems almost remarkable that there are any obserable electrophoretic classes of globulins. In fact that there are such groups suggests a certain economy with respect to some of the characteristics of the plasma globulins, that is, a similarity in structure with minor variations which may influence other properties. The degree to which the study of plasma proteins can be of value in the future undoubtedly depends upon the extent to which fractionation methods can be refined and the extent to which plasma protein changes can be studied by highly specific methods. A case in point, and the only one that comes to mind where a highly specific change in cancer is known, is the increase in serum acid phosphatase in carcinoma of the prostate (Gutman et al., 1938). Of course, this change is not generally indicative of cancer, but is a reflection of the fact that the malignant cells derived from the prostate retain the capacity of normal prostatic tissue to produce large amounts of acid phosphatase. The acid phosphatase, in turn, is a component of the plasma protein which can be measured in a highly specific way, by its enzymatic activity. My feeling would be that the more often such specific changes can be employed in future studies and the more often they can be related to the capacities of particular tissues, the more valuable the studies will be.

Changes in the plasma proteins in carcinoma were recognized as early as 1918, when Loebner showed that the albumin-globulin ratio was decreased. Since that time, many students have confirmed the finding that the albumin is decreased in human cancer and that the globulin is generally increased. In 1928 Starlinger and Winands showed that fibrinogen may be considerably increased in cancer and this finding has been amply confirmed. It is clear that these changes are nonspecific in the sense that they are observed in many other diseases, particularly in wasting diseases. It has not been clear, however, whether such observed changes are only due to the fact that cancer involves a wasting of normal tissue. In the case of gastric cancer, at any rate, it appears that the low serum albumin is not the result of negative nitrogen balance. Studies of Homberger and Young (1948) have indicated that the lower serum albumin persists even in the face of the main-

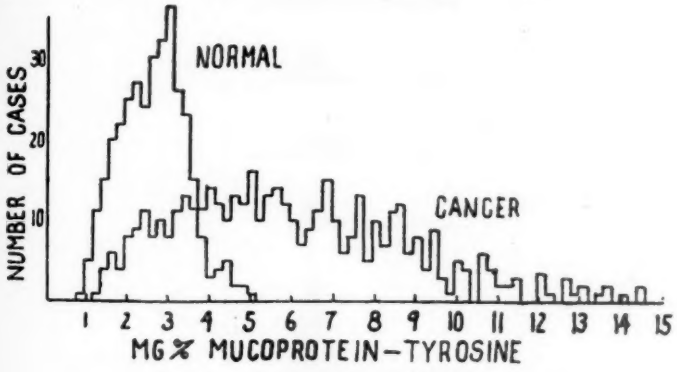
tenance of a positive nitrogen balance. It must be recognized, of course, that a positive nitrogen balance might be found in an individual in whom normal tissue was being broken down, provided that an abnormal tissue was being formed at a sufficiently rapid rate. The normal tissues and plasma proteins might be depleted at the expense of tumor tissue even though a positive nitrogen balance of the body as a whole were maintained. The electrophoretic changes in the serum of patients with cancer as measured at pH 8.5 do not appear to be particularly remarkable or characteristic, but seem rather to present a picture seen in wasting distases of other types. The results of Peterman and Hogness (1948), obtained on patients with gastric cancer show a consistent decrease in albumin, with less remarkable changes in other components. The α_1 globulin tends to be somewhat above normal, or in the high normal range, and the fibrinogen also may be somewhat elevated. In patients with gastric ulcer, however, similar changes are seen. After removal of the tumor, the changes in the globulin are largely reversed in a matter of weeks; but the albumin changes do not reverse themselves rapidly, and even after a year or more the albumin may still be low. In these patients operated for gastric cancer there is a question as to the extent to which gastrectomy interferes with digestion and absorption.

Recently Huggins, Miller, and Jensen (1949), have presented evidence that may be interpreted as indicating that the albumin in the serum of cancer patients is different from that in the serum of normal individuals. This is based upon the observation that the thermal coagulation of serum under specified conditions requires a greater total protein concentration or a greater albumin concentration than normal serum. As another indication of a difference between normal and cancer serum they found the amount of iodo-acetate required to inhibit heat coagulation of a given amount of protein was smaller in the case of the cancer serum than in normal serum. In other words, at a given protein concentration the heat coagulation of serum of cancer patients takes place less readily than that of normal patients, and that may be interpreted as indicating a difference in the proteins of normal and cancer blood. Huggins et al. (1949), feel that the changes are not due to the decrease in

albumin because, when the albumin is estimated by salt fractionation, the same differences appear if the albumin, rather than total protein, is used as the basis for calculation. This is interpreted as meaning a difference in the type of albumin, but I think that until studies are made with albumin isolated from patients with cancer and a difference is shown for purified albumin, one would be left with the possibility of interpreting the results in terms of changes in the relative amount of albumin and globulins. As pointed out by the authors of this study, this change is not specific for cancer but is also observed in tuberculosis and in certain other diseases.

I would like to consider another change observed in the serum of cancer patients, which has a similar lack of specificity. Early studies of quite a group of people including Hahn (1921), Wolf (1921), Burstein (1937), Waldschmidt-Leitz and Mayer (1939), and others have shown that there is a substance with a polypeptide or proteose-like behavior in serum and that this substance increased in the serum of cancer patients. Mayer showed, in 1942, that a substance could be obtained from normal serum which had the same properties, in that it was not precipitated by sulfosalicylic acid and was also non-dialysable, and which was a glyco-protein. Dr. Winzler undertook some studies of this material at the National Cancer Institute with Dr. Burk (1944), and has since continued at the University of Southern California. It is found that perchloric acid offered certain advantages in the separation of this material from other plasma proteins since, as with sulfosalicylic acid and trichloroacetic acid, this material does not precipitate with the other plasma proteins. It is, however, precipitable by tungstic acid and can be precipitated by saturation with amonium sulfate. Material isolated from normal serum (Winzler et al., 1948), has been shown to contain only 7.9% nitrogen. Both on the basis of the nitrogen content and of the biuret reaction carried out in the quantitative way, it appears to be only about 60% protein. It contains 15% of carbohydrate, in addition to 12% of hexosamine, and no uronic acid. It contains 4.2% tyrosine, which makes the tyrosine determination of the material in the perchloric acid filtrates a satisfactory procedure for the analysis of serum. The determination of carbohydrates

in the perchloric acid filtrates provides an even more satisfactory basis for the analysis. There is, as yet, no indication that the material from the serum of cancer patients differs from that of normal serum. Typical values for the tyrosine in the perchloric acid filtrates are shown in Figure 1 (Winzler and



PLASMA MUCOPROTEIN LEVELS IN NORMAL INDIVIDUALS AND IN CANCER PATIENTS

FIG. 1. Plasma mucoprotein-tryosine levels in normal individuals and in patients with cancer.

Smyth, 1948). These are cancer patients selected without respect to the size of the tumor or the severity of the involvement. There is some overlapping of the values in serum from cancer patients and normal individuals, but in general the values are elevated in cancer. Patients with tuberculosis, pneumonia, rheumatic fever, and rhematoid arthritis, however, may show the same sort of increase that is observed in patients with cancer so that we are dealing with a change which is nonspecific.

During the course of study of the methods for isolating this mucoprotein from normal serum, a large number of samples were studied electrophoretically as a guide to the isolation procedure. Figure 2 shows patterns as pH 8.5 of several mucoprotein preparations. It will be evident that they are all heterogeneous, and though all but one of the preparations were made in essentially the same way, there is an inconsistency in

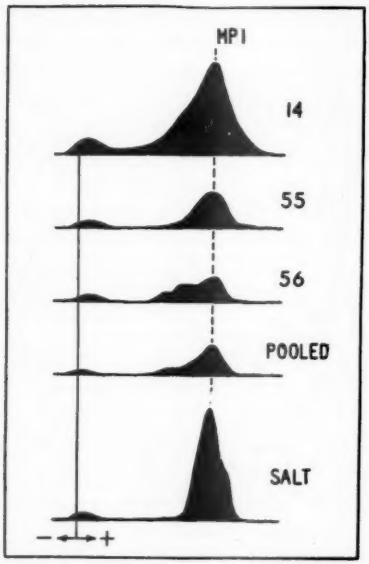


FIG. 2. Electrophoresis patterns of plasma mucoprotein preparations at pH 8.4. All are isolated from perchloric acid filtrates of plasma, except that designated "salt", which was prepared entirely by salt fractionation.

the exact number of components. A large amount of material with a relatively rapid mobility is always seen, and this component (MP–1) is seen in a more homogeneous form in the material prepared entirely by salt fractionation. The "salt preparation" is contaminated with albumin, but contains the component MP–1 in a quite homogeneous form. Figure 3 shows some of the preparations at two other pH's, and the particularly interesting thing is that at pH 4.5 the components are still negatively charged. Even at pH 3 one of the components is still negatively charged, and the iso-electric point of that component is near pH 2. This is, of course, a very acid iso-electric point compared to those of the other plasma proteins.

When we were about at this point in our studies, we were informed by Dr. Peterman of work that she had carried out on serum at pH 4. At that pH it had been possible to demonstrate

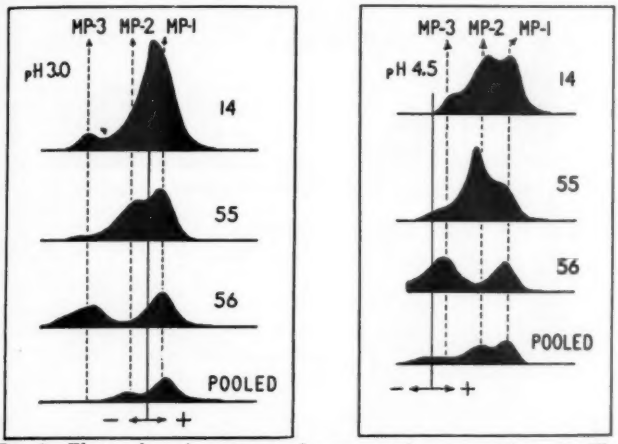
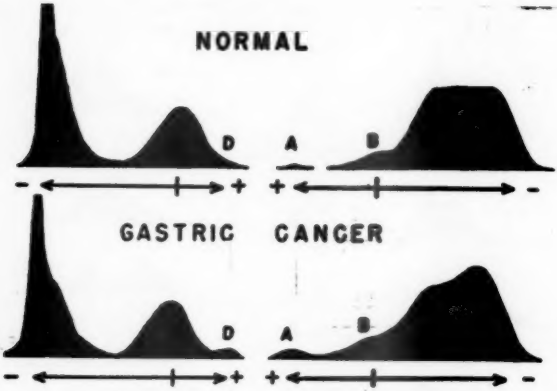


FIG. 3. Electrophoresis patterns of mucoprotein preparations at pH 4.5 and pH 3.0.

an acid component in the serum of patients with gastric cancer as well as in certain other conditions. The nature of these results, which have since been published (Winzler and Smyth, 1948), is shown in Figure 4. In the electrophoresis pattern at pH 4.0 the component which still migrates toward the positive electrode (D and A) is detectable in the normal tissue but increases considerably in gastric cancer. The amount of this acid component is less than 150 mg. per 100 cc. of normal patient's serum, and larger amounts are found in gastric cancer, leukemia, cancer of the lung and Hodgkin's disease; so there is a similar distribution of this component and the mucoprotein in which we have been interested. We decided, upon the basis of the studies with the isolated mucoprotein, that it would be desirable to work at pH 4.5, where the albumin is isoelectric, the globulins move toward the negative electrode and the mucoproteins in the opposite direction. As shown in Figure 5, there is also a component, which we designated as M–2, which appears under these conditions but which is not evident at pH 4.0. We use the boundary ascending to the positive electrode for the analysis because the albumin area corresponds, at least within five or ten per cent to that at pH 8.5. In Figure 6, patterns are shown at pH 8.5 and pH 4.5 of a normal plasma,



Representative electrophoretic patterns obtained at pH 4.0 on the plasmas of normal subjects and of patients with gastric cancer. The rising boundaries are on the left, and the falling boundaries on the right.

FIG. 4. Electrophoresis pattern of serum, ascending and descending boundary at pH 4.0. From Petermann and Hogness. (1948).

with and without the addition of mucoprotein. The mucoprotein preparation was that made entirely with ammonium sulfate, and contained only MP–1 and albumin. It is evident that this mucoprotein preparation increases α_1 at pH 8.5, and the M–1 component at pH 4.5. In the same figure we have the electrophoretic pattern of serum from a patient with cancer, with and without the addition of mucoprotein isolated from a perchloric acid filtrate. In this case, there is a rise in the amount of M–1 and also a rise in the amount of M–2. In the mucoprotein preparations we also had a component migrating between M–1 and M–2 appearing in many preparations. We have never observed such a component in serum itself, and there is still considerable question in our minds as to the relation between the slow component in serum and the slow component in the isolated material; but we believe that the

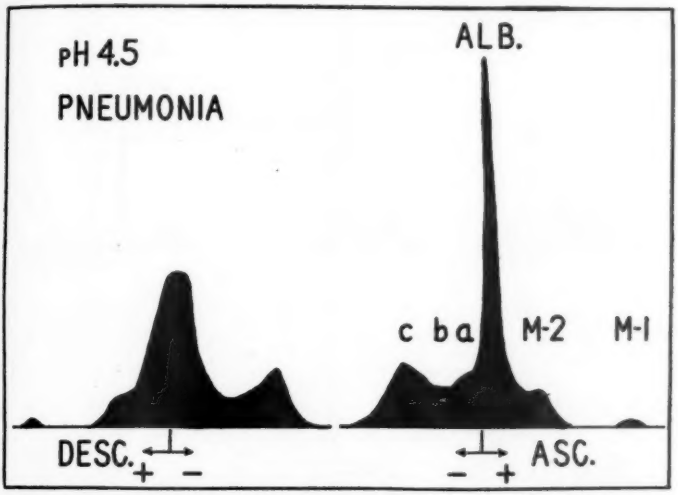


FIG. 5. Electrophoresis pattern of serum at pH 4.5, ascending and descending boundaries.

M–2 component of serum probably is also a mucoprotein. In Figure 7 are the patterns at pH 4.5 of serum from three patients with pneumonia and three patients with cancer, in all of which there is an increase in the mucoprotein. The mucoprotein-tyrosine values in milligrams per 100 cc. (MPT) is indicated in each case. If one attempts to correlate the MPT values with the electrophoretic patterns, it is apparent that the M–1 fraction is increased about the same amount in all of the cases except one in which the MPT is relatively low. The amount of M–2, however, does not appear to bear any constant relationship to the MPT value or to the amount of M–1, though we have not observed an increase in M–1 without some increase in M–2. We are as yet unable to offer an explanation of the significance of M–2. Neither do we have any very good idea of the source of this material. We intend to pursue that problem, but it is certainly clear now that the polypeptide or proteose, as it originally was called, is a mucoprotein. It does rise when cancer is produced in experimental animals, and in patients with cancer, but it also rises in a great many other diseases and so is not specific for cancer. Further studies of this component would

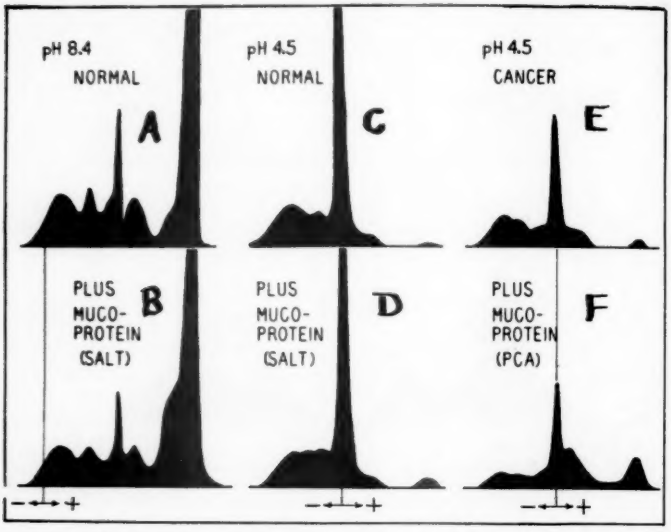


FIG. 6. A. Normal plasma at pH 8.5, descending boundary. B. Normal plasma with mucoprotein added' at pH 8.5, descending boundary. C. Normal serum at pH 4.5, boundary ascending to positive electrode. D. Same, with mucoprotein added. As in B, mucoprotein contains only MP-1. E. Serum from cancer patient at pH 4.5, boundary ascending to positive electrode. F. Same, with mucoprotein added—Mucoprotein contained MP-1, MP-2, and MP-3.

only be likely to have value in helping us to understand something of the metabolic changes going on.

There is one specific type of neoplastic diseases which I would like to mention in closing in which there are known to be rather specific changes in the plasma protein. We have been studying patients with multiple myeloma for about two years now and have accumulated a good deal of information. Our results, however, will actually add little to the results recently reported by Adams, Alling and Lawrence (1949), as our findings have been essentially the same as theirs. By far the most common finding, aside from the histological changes on which the diagnosis is based, is an abnormality in the plasma proteins. This is generally a striking increase in some component with a mobility the same or slower than that of β-gloublin

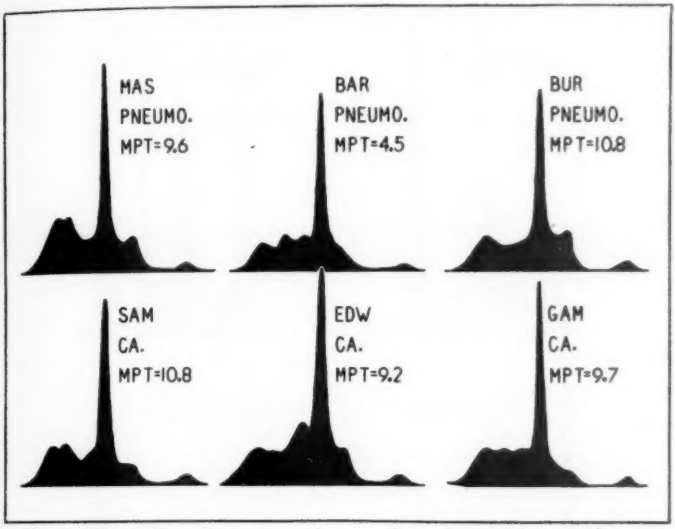


FIG. 7. Electrophoresis patterns of serum from three patients with cancer and three with pneumonia, at pH 4.5.

(Figure 8). Components are not uncommonly seen which are essentially immobile at pH 8.5 (Figure 8). Hyperglobulinemia of a rather characteristic sort is reported for some 70% of cases, as against 50% or less showing Bence Jones proteinuria. Occasionally one encounters a case in which there is no hyperglobulinemia, but the patterns in these cases are generally not strictly normal (Figure 8). Abnormalities may include a decreased γ-globulin, or the presence of a component (seen in the serum) with a mobility about that of fibrinogen. We have most often found such a component to correspond in mobility to Bence Jones protein found in the urine, and it may be presumed that urinary loss prevents hyperglobulinemia due to this component. However, there may be both hyperglobulinemia and Bence Jones proteinuria, often due to demonstrably different components with respect to electrophoretic behavior. Such a situation, as well as the value of studies at more than one pH are illustrated in Figure 9. Serum from a patient with multiple myeloma showed, at pH 8.5, a typical increase in the β-globulin region. The urine contained Bence Jones protein,

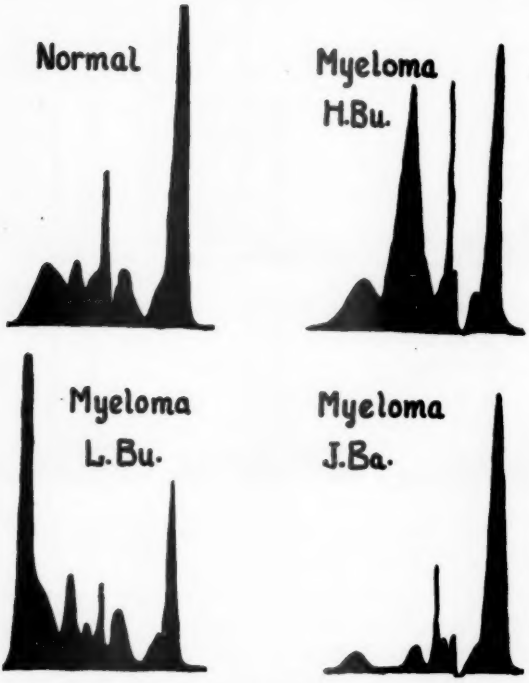


FIG. 8. Electrophoresis patterns, at pH 8.5, of normal plasma, and of sera from three patients with multiple myeloma. The two sera with hyperglobulinemia represent approximate extremes of mobility of the abnormal components. The case without hyperglobulinemia represents a type less frequently seen.

and electrophoresis at pH 8.5 showed this to be nearly homogeneous and to have a mobility a little slower than the bulk of the abnormal globulin in the serum. This difference between the abnormal serum globulin and the urinary protein was confirmed by electrophoresis at pH 4.5, which also shows that the abnormal globulin of the serum consists of at least two components. We have often noted the same behavior at pH 4.5 when the abnormal peak is in the β-globulin region at pH 8.5. When the abnormal globulin has a mobility nearer that of γ-globulin it is generally observed to be a much sharper peak

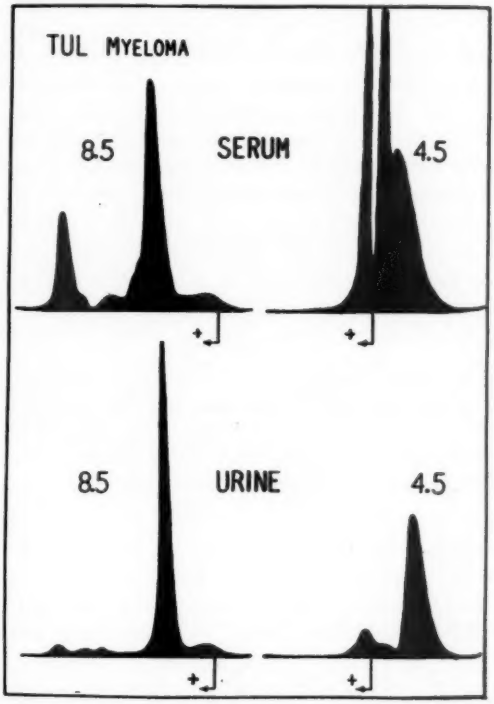


FIG. 9. Electrophoresis patterns of serum and urine from a patient with multiple myeloma, at pH 8.5 and pH 4.5, demonstrating that the urinary protein differs from the serum components responsible for the hyperglobulinemia.

(more homogeneous), and to appear as a single peak in the electrophoresis pattern at pH 4.5.

In conclusion, the changes in the plasma proteins which occur in neoplastic diseases do not as yet appear to be specific but may be observed in other diseases in which wasting is an outstanding feature. The only situation in which changes in the plasma proteins appear to be particularly useful as a diagnostic adjunct is in the case of multiple myeloma where the appearance of a sharp peak corresponding to an abnormal globulin lends confirmatory evidence to a diagnosis of multiple myeloma.

Note

The electrophoresis studies have been carried out in the laboratories of the Los Angeles County General Hospital, and were supported by research grants from the United States Public Health Service. We are indebteded to Drs. Gurth Carpenter and E. M. Evans for assistance in obtaining material on the patients with multiple myeloma.

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THE USE OF GENETICS IN CANCER RESEARCH*

C. P. Oliver

A statement which is often made is that "cancer in the human is not hereditary but the tendency to cancer my be hereditary." Geneticists readily agree that cancer, as such, is not inherited. However, cancer is not unique in that respect. One never inherits any trait, not even a physical characteristic such as eye color. Genes, which make a hereditary trait possible, are transmitted from a parent to child. A combination of genes gives an individual a particular potentiality, tendency, or susceptibility. Unless the proper structure or the environment specific for that gene action is also present, the hereditary tendency will not be expressed.

The statement referred to is unfortunate because it is likely to create an unnecessary fear of heredity. It suggests to many individuals that if a hereditary basis for cancer is proven, a member of a "cancer family" has little chance to escape the disease. Yet, experimentalists have been able to demonstrate that heredity tendencies can be controlled. In some organisms it is possible to prevent a hereditary trait from occurring even though an individual has the genetic constitution for the trait. At present, we are not in such a favorable position with respect to human traits, but progress can be expected.

We are not to imply that a hereditary tendency for all types of cancer in human beings has been proven. Active research on this question is now being made in several centers. A group at The University of Texas is carrying on an investigation of the genetics of human breast cancer. The project is made possible by a grant in aid from the American Cancer Society through the Committee on Growth to The University of Texas and through the co-operation of Dr. Dudley Jackson of San Antonio, who with a grant from the National Cancer Institute, U. S. Public Health Service, makes available to us data which can be followed in family analyses.

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*The University of Texas, Austin. Presented at the 1949 Cancer Conference, M. D. Anderson Hospital for Cancer Research.

Nucleus, Cytoplasm, and Cancer

The study of classical genetics is concerned primarily with segregation results and with determining the probability that a trait will appear in offspring whose parents have known genotypes. The behavior of the nucleus of the cell and more particularly of the chromosomes which carry the genes are important in the study. Geneticists also are interested in the physiological process of development. Attention is paid to agents which affect the hereditary potentiality and thus change the probability that a trait will develop. The cytoplasm, the cytoplasmic inclusions, and the interrelationship between the nucleus and cytoplasm are factors, all of which must be taken into consideration in the modern study of genetics.

The opinion that the cytoplasm is closely associated with cancer occurrence has many proponents. This belief does not necessarily discredit the idea that genes in the nucleus also play an important role. Genes are known to affect some components of the cytoplasm. For example, plant plastids occur in the cytoplasm. They are transferred from mother cell to daughter cells through the cytoplasm as a medium. The presence of pigment in these plastids may be controlled by some agent acting upon the cytoplasm. Pigment development may be affected also by genes located in the chromosomes.

Both the cytoplasm and the nucleus are responsive to outside agencies. Certain agencies act as mutagens in that they cause mutations in the genes and chromosomes which result in hereditary modifications. Other agencies act as carcinogens and cause malignancy to develop. Strong (1945) has reported that the carcinogen, methylcholanthrene, also causes mutations in mice. Carr (1947) reported that the carcinogen, dibenzanthracene, acts as a mutagen in mice. Demerec (1948) reported that some, but not all, carcinogenic hydrocarbons can be used to induce lethal mutations and chromosomal aberrations in Drosophila. Irradiation is an effective mutagenic agent and can cause cancer, but the malignancy occurs only if the intensity is great enough to cause heavy damage to the cytoplasm. Nitrogen mustard is mutagenic (Auerbach, 1947), but it is more than a weak carcinogen (Boyland and Horning, 1949).

Demerec observed in his study of the mutagenic effects of hydrocarbons that individuals in the same experiment varied in their response to the mutagen. This difference in behavior he attributed to possible genic differences of the individuals. Demerec suggested that irradiation and chemicals cause gene mutations in the somatic cells and that these mutations probably bring about cancer. Even if somatic mutations are the first step in cancer occurrence, other factors require explanation. Various strains of animals differ in their tendency to develop cancer. Certain cell-types in a strain are more likely to show the change to cancer than are other cell-types. One strain of mice, for example, has a strong tendency to develop lung cancer. Animals from another strain are not so likely to have lung cancer, but they have other tissues which show the cancer tendency. These strains have been developed by breeding and selection for susceptibility. It seems, therefore, that the genes in the nucleus have an important part in cancer susceptibility and in determining the tissue which will respond most readily to the cancer activator.

Genes as Factors in Mouse Tumors

The knowledge about cancer development in mice has progressed to the point that most investigators recognize a heredity susceptibility of mice to mammary cancer, lung cancer, and leukemia. In the early studies, however, a genetic basis for cancer occurrence was not so readily detectable. Colonies of mice differed with respect to the incidence of cancer among the animals. Some colonies remained free of the disease. Others had from a few to many cancerous members. Affected animals in colonies might have only one site or more than one site involved. As data accumulated, it became evident that cancer occurred only in animals kept in certain cages. Contamination and infection were disproven by experimentation. Workers began to conclude that cancer has a hereditary basis. Little recognized that the study of the genetics of cancer in mice could be approached scientifically only if homogeneous strains were available. Strains were developed by breeding and selecting for susceptibility and resistance to cancer. Strong has been responsible for many of the homogeneous strains now used.

(For a more detailed history of this development see Heston 1945).

Inbred strains have been developed, each of which has its characteristic tumor incidence and site of tumor. Strain A, for example, has a high proportion of pulmonary adenomatous tumors. Breeding females also have a high incidence of mammary tumors. Strain C3H has a high incidence of mammary tumors in both breeding and virgin females. Strain dba also is a high mammary cancer group. On the other hand, C57,-black has a low mammary cancer incidence. Less than 1% of the breeding females develop mammary tumors.

These highly selected strains make genetic studies possible. Pure genetic strains are also valuable for accurate measurements of the effect of non-genetic factors on the occurrence of cancer. If sub-lines have been separated for a long time, mutations may have occurred so that the lines cannot be used for comparative purposes. Mutations, though, are too infrequent to invalidate repeated experiments using animals from one colony.

Lung cancer in mice, as shown by Lynch (1926), Bittner and Little (1937), and Bittner (1948) is strongly genic in nature. Heston (1940) concluded that more than one pair of genes are associated with lung cancer susceptibility. Extrinsic agencies may act as incitors to cause an earlier manifestation of the disease but do not increase the tendency for cancer to occur.

Leukemia in mice has a genetically determined susceptibility. Hogreffe (1948) reported that animals from a strain with high incidence mated to resistant animals produced progeny with an intermediate incidence. The F_1 leukemic animals developed the disease at a later average age than that of the high leukemic parental strain. Backcross tests of the F_1 to the parent strains gave progeny which had an incidence and onset age approaching that of the respective pure-line strain used in the backcross mating.

Mammary cancer in mice develops in females which have a genetic constitution for susceptibility. This conclusion is substantiated by hybridization and linkage tests. The reports are numerous and have been summarized by Heston (1945, 1948). Susceptibility seems to have a dominant inheritance and probably involves more than one gene pair (Bittner, 1944).

Susceptibility is only one of three agents active in the development of mammary cancer. The other two are a hormonal influence and a milk agent (Bittner, 1942b). A hormonal effect was discovered by the study of strain A and strain C3H mice both of which have a high mammary cancer incidence among breeders. Strain A, however, has a low incidence, among virgin females, whereas virgin C3H females remain a high strain. Breeding tests show that the hormonal difference has a genetic basis. If an A animal is mated to a C3H animal, irrespective of which strain supplies the female, the progeny will have a high cancer incidence among the virgins. Segregation occurs in the F_2 (Bittner 1939; Heston and Andervont 1944).

The third agent effective in causing mammary cancer in the mouse is supplied by the milk (Bittner 1936, 1942a). The presence of this agent can be demonstrated by reciprocal matings. If a high strain female, A or C3H, is mated to a low C57, and the offspring are nursed by the high strain mothers, the progeny have a high cancer incidence. In the reciprocal test, the progeny are nursed by their low strain mothers and have a low cancer incidence.

Foster nursing also has been used to show the efficacy of the milk agent. A female from the high A strain which is removed from her mother at birth and is then nursed by a C57 black female is not likely to develop cancer of the breast. A low-cancer strain female nursed by an A strain mother will probably develop a mammary tumor. The milk has, therefore, a definite part—at least as an activator or carcinogen. The milk agent is less effective in older mice, but this resistance can be overcome by the use of heavy doses given to susceptibles.

Strains of mice vary in their ability to propagate and transmit the milk agent. Every high-incidence strain has that ability. Low-strain C can also propagate and transmit the agent (Andervont 1945). Low-strain C57 black females will respond to the milk agent received by foster nursing. They can transmit the agent (Andervont 1945) so that susceptible animals will develop cancer. In matings of high to low strains, the animals in later generations vary in their ability to transmit the agent (Heston, Deringer, and Andervont, 1945). Hybrid females were backcrossed in tests for differences in ability

to transmit the agent. A high-strain C3H female was mated to a low-strain C57 male. Some F_1 females were backcrossed to C3H males; others, to C57 black males. Female offspring of the C3H backcross could transmit the agent. Females from the other test were not so proficient. Heston believed that the difference is traceable to the genes from the males used in the backcross mating.

Heston (1948) also reported that some females develop a tumor without the benefit of the milk agent. A C3H sub-strain (C3Hb) was bred brother-sister. This strain has a cancer incidence of 24 per cent, although the milk agent was not transmitted by nursing. The cancerous mice occur somewhat sporadically among the descendants. Heston suggests that the milk agent acts as a carcinogen which increases the probability of mammary cancer occurrence, in a manner similar to the activators of lung tumors.

Genetics of Human Cancer

The study of the genetics of human cancer will be complicated and very laborious, but it is not an impossible task. The heterogeneity of the human race causes difficulties as great as those encountered in the early studies of cancer in mice before inbred and homogenic strains were developed. Persons having a mixture of genotypes and living under various environmental conditions are grouped together according to phenotype or appearance. Data to be used for comparisons with the cancerous patients are difficult to collect with accuracy and completeness. The morbidity cancer rate is not readily available for the several populations or sub-populations. A good control group for comparative purposes is difficult to find. On the basis of our knowledge of experimental genetics we must also recognize that a condition in one person may have a genetic basis, whereas in another it may occur due to non-genetic factors.

Two methods are available for the study of genetics of human cancer: a comparative study of monozygotic and dizygotic twins, and an analysis of family histories which are made complete and accurate.

Cancer in Twins

Macklin (1940) collected from the literature the reports of cancer occurrence in twins and added 15 other cases about which she had knowledge. These data are arranged in tabular form (Table I).

TABLE I

Cancer Occurring in Like-sexed Twins

Twin Type	No. Twins	Both Twins with Tumors		Both Twins with Tumors of Same Site	
		Number	Percent	Number	Percent
Monozygotic	53	32	60	31	58
Dizygotic	27	12	44	6	26

The observed difference between the proportion of monozygotic twins and dizygotic twins, both of whom have cancer, is not striking. Of considerable interest, however, is the greater tendency for monozygotic twins to have the same site affected. The concordance for tumors of the same site was approximately twice as high in monozygotic as in dizygotic twins. The respective percentages were 58 of all monozygotic twins and 26 of all dizygotic pairs. In familial cancer, the practice is to refer to similarity of site of cancer among affected relatives. If this is done for the twin-pairs where both members of a pair are affected, 97 per cent of monozygotic twins and 50 per cent of the small number of dizygotic twin showed concordance with respect to site. The monozygotic twins also tend to have cancer occurrence at the same relative ages. Macklin reported that the average variation in age at onset for monozygotes was 1.2 years in contrast to 9.5 years for dizygotes.

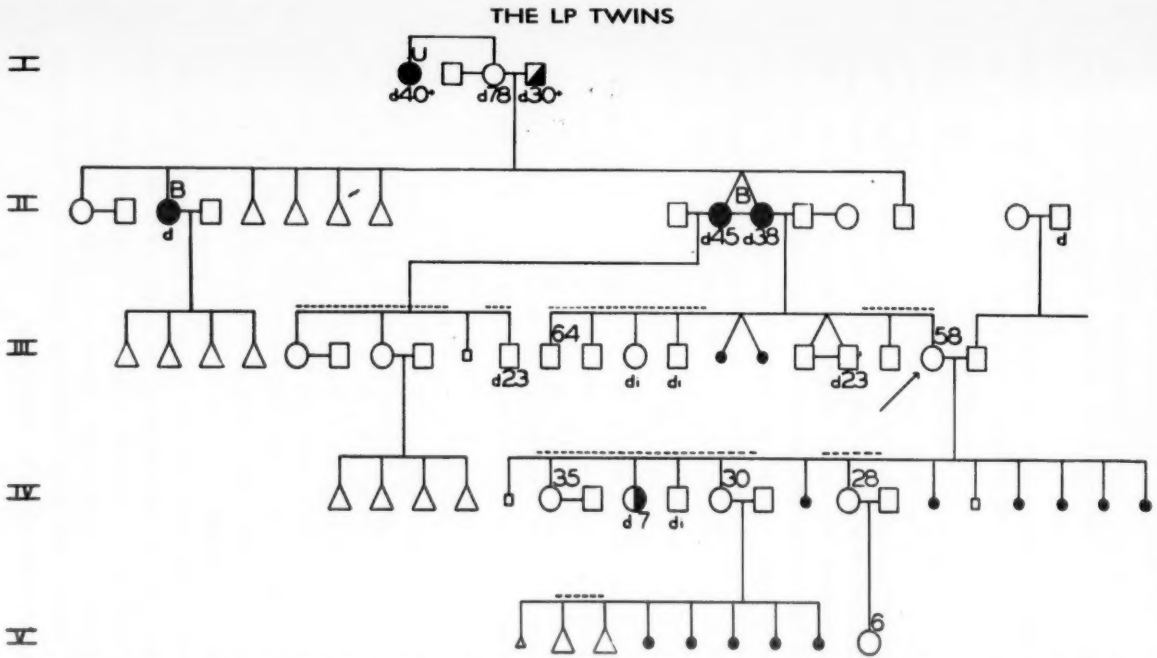
Busk, Clemmesen, and Nielsen (1948) collected data on cancer occurrence among 185 twin pairs. Seven of 52 monozygotic pairs (15.4 per cent) and seven of 133 dizygotic twins (5.3 per cent) were in concordance. Similar sites were affected in four of the seven monozygotic pairs and in only one of the seven dizygotic pairs.

Three sets of twins with both members of a pair affected with breast cancer have come into our study. One pair is considered by members of the family to have been fraternal or

dizygotic twins. They were very dissimilar in appearances. Both died from breast cancer. Another pair, the *LP* twins, most probably were monozygotic in origin. Both have died from breast cancer. The third pair, the *DS* twins, are living. Tests substantiate the family's opinion that they are monozygotic twins.

The pedigree chart for the *LP* twins is shown in Figure 1. One of the twins in generation II died at 38; the other, at 45 years of age. Cancer of the breast was reported to have been the cause of death. One sister (B in generation II) of the twins also died with cancer of the breast. The other sisters and brothers of the twins have remained free of cancer. A maternal aunt of the twins, reported to have had cancer of the uterus, died in her early forties. She had a large family. The father of the twins is reported to have had a brain tumor. Two granddaughters of one of the twins are to referred to later. In the family chart they are identified by the symbols d7 and 28 in generation IV. Verifications of these cancer cases have been practically impossible.

Because of reports concerning child-bearing and breast cancer occurrence, the pregnancy histories of the members of the family are of interest. The cancerous sister of the *Lp* twins had four children. The non-cancerous sister shown in the pedigree chart had no pregnancies. The twin who died at age 45 had four pregnancies; the other twin had eight. The twins' mother had eight pregnancies. She died at age 78 without having developed cancer. She, therefore, lived 33 years past the age of death of one twin, and 40 years past the age at death of the other. The twin who died at age 45 had two daughters who have remained free of cancer to date. One of these daughters had no pregnancies, and the other has had four. One daughter of the other twin lived past childhood. This person is marked with an arrow in Figure 1. Although she is free of cancer at an age of 15 years past the age at which her mother died, the daughter has a definite fear that she will develop cancer. This daughter has had 13 pregnancies but only five live births. The first-born is a daughter, now age 35, who has no children. The second was a daughter who died at age 7, reportedly with Hodgkin's disease. A son died at the age of three weeks. A



LEGEND FOR FIGURE 1. Familial chart of the LP twins. Symbols used: squares represent males; circles represent females; triangles represent persons whose sex has not been definitely determined. Large solid circles are females with cancer of the uterus (U) or of the breast (B). Half solid circle or square represent possible cancer as indicated in text. Letter "d" indicates deceased with "di" for death in infancy. Numerals indicate age, if living, or age at death if with "d". Broken lines above sibship line indicate children who were breast-fed.

daughter, now age 30, has had two live births, one stillbirth and five abortions. The fifth, a daughter, age 28, has had one pregnancy.

Information about breast feeding is known only for the part of the family coming through the twin who died at age 38. This twin breast-fed all of her children except the male twin-pair. Her daughter, shown by the arrow, breast-fed all of her children. The granddaughter, marked by age 30 in generation IV, breast-fed both children. The granddaughter, marked by age 28 in generation IV, has reverted nipples and could not breast-feed her only child. Pernicious anemia and tuberculosis are reported in several members of the family.

A brief report of the *DS* twins has been given (Jackson et al.). The monozygotic origin of this pair seems well established. They belong to blood group O and types M and Rh positive. Their PTC taste threshold, body build, eye color and hair are identical. Finger prints and palm patterns are similar.

First evidences of pathologic conditions of the breast occurred at approximately the same age in the *DS* twins. Through a period of years, one and then the other reported for breast examinations, and the historical examinations gave evidence of similar types of hyperplasia. At age 47, one of the twins had a lesion reported to be precancerous, and a simple mastectomy was performed. Within three months, the other twin had an adenocarcinoma; and in the surrounding tissue the pathology was similar to that observed in her twin. The similarity of the breast hyperplasia in the twins is striking. The age at onset and progress of the disease in the two have been practically the same. Although the similarity is not proof that heredity was a factor in the development of the pathologic condition, it is what might be expected in monozygotic twins if heredity is involved.

In contrast to the *Lp* twins, the *Ds* twins were relatively infertile. One member became pregnant once, but this ended in an abortion. The single pregnancy in the other produced a daughter. This twin was unable to produce milk for her child. Both twins stated that they wanted other pregnancies but were unable to have them.

The mother of the *DS* twins had six pregnancies resulting in one abortion, one twin birth, and four single births. She breast-fed all of her children except the twin who has the daughter. An attempt was made to get this twin to nurse. Among the siblings of the twins, an older sister has had one breast removed. The hyperplasia is reported to have been similar to that of the twin with the precancerous condition. A younger sister has mycosis fungoides. An older brother died at age 40. A younger brother is living and free of cancer. A paternal uncle has had cancer of the stomach. No other relative is reported to have had cancer, but all of the paternal and maternal uncles and aunts died at an early age. Diabetes is prevalent in the family record. The mother of the twins was diabetic and died at age 39. Two of her sisters had diabetes. The younger brother of the twins is now under treatment for diabetes.

Familial Cancer

In spite of the heterogeneity of the human population, data collected by many investigators supply evidence that many cases of cancer have hereditary basis. The complete story will be unravelled only after detailed familial studies are completed, including interviews with "normal" relatives of cancer patients. Information collected only from patients will be wholly inadequate for a genetic interpretation.

Some forms of cancer susceptibility are now recognized to be strongly hereditary. Xeroderma pigmentosum, a hypersensitivity of the skin to sunlight, has a tendency to become malignant. Its method of inheritance has been determined. The susceptibility is due to a recessive incompletely sex-linked gene-pair. The genes are located on the homologous parts of the X- and Y-chromosomes in the affected male, and on both X-chromsomes in the female. Consequently, there is a tendency in a given family for only one sex to be affected. In some families, crossing-over between the homologous parts of the X- and Y-chromsomes make it possible for both males and females to be affected. As may be expected with a rare recessive trait, a large proportion of parents producing children with the trait are blood relatives.

Retinoblastoma has a dominant inherited tendency according to most investigators. Falls (1947) reported a pair of twins both of whom were affected, and referred to two other pairs of twins in which this holds true. Familial records indicate that hereditary tendency for this cancer is transmitted directly from parent to child, but the gene does not always become manifested.

Occupational cancers also occur. Chimney sweeps have long been known to be prone to develop scrotal cancer. Workers with aniline dyes have a high incidence of bladder cancers. Heredity in these cases will be at the most only of secondary importance.

Some families seem to have a hereditary type cancer, but from the available data it is not always possible to determine any specific method of inheritance. These families show a relatively high concentration of affected members. Of even greater importance is the tendency for the cancerous relatives to have a particular site affected, as though certain cell types were more responsive to carcinogenic agents acting as activators or incitors.

A family history with a concentration of cancer has been reported by Hauser and Weller (1936). In this family, 174 members were 25 years of age or older, 25 being the earliest age at onset for any member. There were 43 cancers in 41 persons. In 26, the disease involved the stomach; in 15 the uterus; and one each of the breast and face were reported. In percentage, 23.6 per cent of the 174 had one or more cancer; 14.9 per cent had stomach cancer. Of the 43 cancer sites, 60 per cent were of the stomach, 35 per cent were uterine. One might argue that Warthin and, later, Hauser and Weller happened to find a "cancer family." It is also possible that they have shown what can be discovered if an investigator makes a complete study of one family.

Bargen, Mayo, and Giffin (1941) studied the proportions of affected relatives of 176 patients with malignacy of the large intestine, and those of 176 arthritic patients. They found that the cancerous relatives of the patients with cancer of the intestine were more likely to have cancer localized in those regions than cancer at another point. They also reported a

higher incidence of cancer among the relatives of patients with malignancy than among those of the noncancer patients, the respective percentages being 26 and 6.

Thomas, Dockerty, and Waugh (1948) studied multiple cancers of the large intestine, which included all colon-rectal cancer cases. Multiple synchronous cases were 91; asynchronous, 37. Among the former 26 (28.6 per cent) had cancerous relatives. These relatives had 12 cancers of the large intestine, 5 of the stomach, and 33 miscellaneous. The familial incidence in the asynchronous cases was even greater, being 54 per cent. Among 35 affected relatives, 11 had cancer of the colon, 4 of the stomach, and 20 elsewhere.

Other investigators (including Wassink, Martynova, Jacobsen, Videbaek, Smithers, and Penrose) have reported concentrations of sites in families. Patients and their affected relatives have the same sites affected more frequently than would be expected on comparison with other control groups. In spite of such concentrations, some investigators refuse to accept the data as strong evidence that heredity is a factor in the occurrence of these cancers. They seem to believe that the high cancer incidence in the families is merely an expression of chance that with cancer occurring so frequently in the general population some families can be expected by chance alone to have several affected members. One point which we will emphasize is that more "cancer families" will be discovered if intensive studies of families are made, such as that made by Warthin and later added to by Hauser and Weller.

It is true that most of the family-history studies face at least two plausible criticisms. One of these is the lack of adequate control group. The other is concerned with the methods used for collecting the data.

An investigator finds it difficult to select a proper control group for his study. The reported incidences of cancer in the control families have been too low when compared with the expected incidence, based upon the population rate. Penrose, Mackenzie and Karn (1948b) attempted to get around this criticism by comparing the data of reported cancer among the deceased relatives of 510 breast cases with the expected number if those relatives were subject to the mortality of the general

population. Penrose reported that the number of propositi's mothers and sisters who died from breast cancer was definitely higher than the expected rate. The number of deaths from other malignancies was just about what would be expected if the relatives were subject to the general mortality rate.

In our own studies of breast cancer, we use for comparative purposes breast cancer patients, benign breast tumor patients, and also a non-tumor control. The non-tumor control cases are persons who are under medical care. This choice is made deliberately because of our belief that a sick person is less likely to emphasize the good health of his family. All data about control propositi and their relatives are collected as painstakingly as are the data about the patients with cancer or benign tumors.

A second criticism of the reports showing concentration is that too much dependence has been placed on a single interview with each patient, in some of the studies at least. It is very essential that the investigator work with the family. Every reported case of cancer among the relatives must be verified, but it is just essential to check the data about relatives who are reported "not cancerous." Normal relatives should be asked for information about cancer cases not reported by the patient. By this method, one quite frequently determines that a supposedly sporadic case is not sporadic. The patient seldom knows about every cancerous relative, even among his close relatives.

In the present (Texas) study, among 117 middle-class, white breast cancer patients, approximately 50 per cent of the women at the time of the interview knew of relatives with cancer. Only about 20 per cent reported one or more relatives with breast cancer. Among the cancerous females reported by the patients, approximately 40 per cent had breast cancer. These percentages approximate those determined for a group of 201 indigent patients reported by Oliver et al. (1946). They differ slightly from data collected by other investigators.

The data are changing rapidly as the patients' relatives are being asked for information about the family. The number of solitary cases is decreasing and the number of affected members of a family is increasing. A few cases showing how the data are changing will be cited.

When patient JC 134 was interviewed, she knew about four relatives with tumors. As a consequence of continued follow-ups, the number of affected blood relatives has now been increased to 23 and will probably go even higher. An attempt is being made to verify each reported cancer. The data on this family are given in Table 2, which shows the number of cases

TABLE II

Additional Evidences of Tumors in JC 134 Family Collected by Follow-ups

Date Data Added	Number of Cases Added	Reported Site of Tumor
11- 6-48 (Patient)	1	Breast (adenocarcinoma)
11- 6-48 (Patient)	4	Uterus, sarcoma, seminoma, skin
2- 7-49	3	Breast, intestine, skin
2-11-49	6	Breast (2), skin (4)
3-16-49	4	Bladder, face, arm, intestine
4-11-49	1	Neck
4-22-49	3	Face, uterus, stomach
5- 6-49	1	Breast
	23	

reported and the site of the tumors reported by additional interviews with members of the family. The information collected on May 6, 1949 (the last date item in the table) came from an affected aunt. None of her sisters or nieces knew that she had had a mastectomy.

In another family, JC 338, the propositus knew about 10 affected relatives. Nine of her relatives gave information about 19 more blood relatives with tumors and also supplied data about some "in-laws" who were affected. In a third family, JC 44, the propositus reported that she had no affected relative, but relatives have given additional data about four affected members. Obviously, there are some persons who know of no cancerous relatives and the relatives themselves report no other cancer in the family. The method of study, however, seems essential for accurate interpretation concerning genetic factors effective in cancer occurrence.

Another method used to assure more accurate and complete data is to record names of all relatives of cancer patients on relative cards so that new data in the future can be linked to

families. It is then possible by cross references to connect some propositi into family groups. An example of this is found in our Cases J 8, 17, 98, 145, 170. Case J 170 is related by blood to J 8 and J 17 but only by marriage to J 98 and J 145. The kinship has, to date, thirteen members reported to have had tumors. At the time of the interview, case J 8 knew about one cancerous relative. Cases J 17 and J 170 reported no cancerous relative. Patient J 98 knew about five affected relatives and J 145 knew about none. It is of interest that these five were patients of one surgeon.

Fators Possibly Affecting Cancer Susceptibility

Among those cancers which tend to show concentration in some families, certain factors or agencies other than heredity seem to play an important part. Marital status and pregnancies, economic status and occupation, race, and age may affect the potentiality. Groups of breast cancer patients may differ considerably with respect to those factors.

The marital status and pregnancy histories for two groups of white patients are shown in Table III. The column headed

TABLE III

Pregnancies of Two Groups of White Patients with Breast Cancer

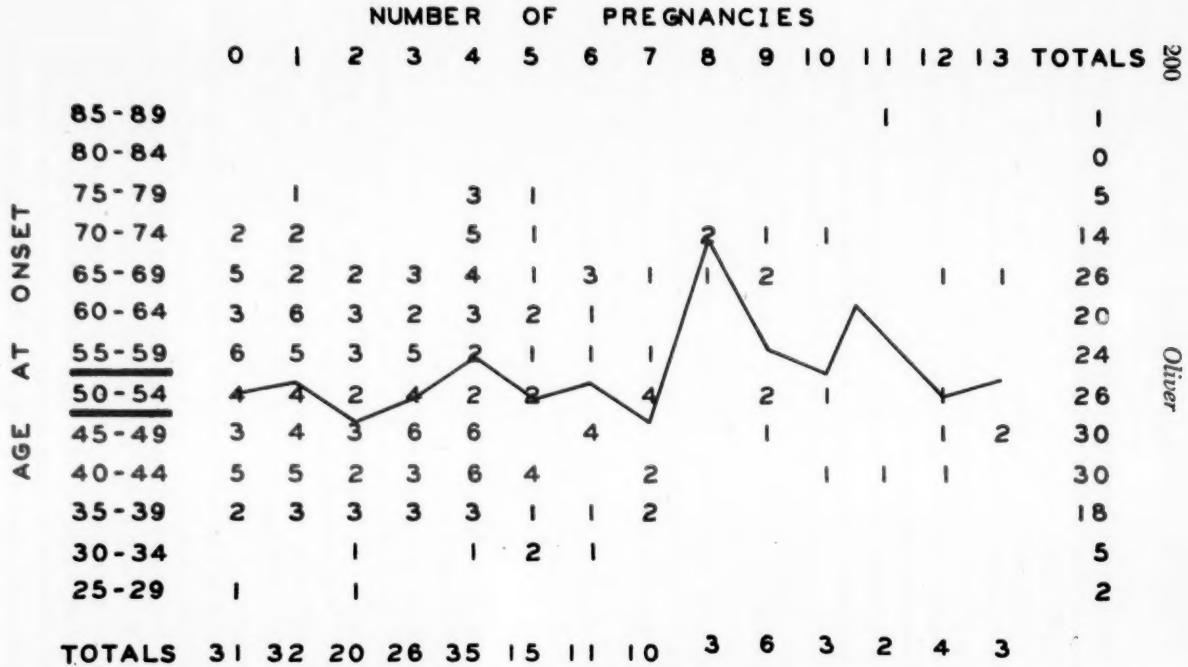
	201 pt 1946 Report	117 pt Present Study
Per cent spinsters	7	13
Per cent childless	15	33
Per cent with pregnancies		
1 to 7	74	64
8 and over	10	3
Average no. pregnancies if at least one	4.2	2.9
Average no. births if at least one pregnancy	3.7	2.7
Average no. births by mother	6.6	5.7
Average age at onset in years	53.4	52.2
S. D. years	11.99	12.75
S. E. years	0.85	1.17

"201 pt." refers to the patients reported by Oliver et al. (1946). These women were indigent patients, for the most part from rural or small urban communities. The column headed "117 pt" refers to private patients of one surgeon. These women belong primarily to the middle-class. The numbers of patients

are small and are being increased. The middle-class group in comparison with the indigent patients has the higher proportion of childless members and also the lower proportion of women with a large number of pregnancies, eight and more. The middle-class group has the smaller average number of pregnancies and of live births, as did their mothers. For comparison, Penrose et al. (1948a) reported that 20 per cent of all patients were childless.

Marital status and fecundity seem to have an important part in breast cancer occurrence (Dorn, 1943; Duffield and Jacobson, 1945). Spinsters face a greater danger than do married women. Childless married women have a relatively higher death rate than do women with children. It has been suggested that increased pregnancies will have a beneficial effect on women with respect to breast cancer occurrence. However, in the study of 201 breast cancer patients, referred to in Table III, no relationship between number of pregnancies and age at onset of cancer was discovered. The distribution of these patients according to number of pregnancies and age at onset is given in Figure 2, which is a modification of the figure used by Oliver et al. (1946). The average age at onset for each pregnancy group, from zero to seven does not differ significantly from the average age for the total group, 53.4 years. Only 21 patients had from eight to thirteen pregnancies, and the number per pregnancy group is too small for critical analysis. The whole group of 21 had an average age at onset not significantly different from those with fewer than eight pregnancies. The average ages at onset of cancer for the indigent and middle-class groups given in Table III do not differ significantly, although the two groups differ with respect to fecundity.

In the report on the number of pregnancies and age at onset for the 201 patients, attention was called to the probably bimodal distribution of ages at onset. One modal class occurred in the 46–47 age group and another in the 67–68 age group. Number of pregnancies apparently does not account for bimodal distribution, if that is a true phenomenon. Jacobsen (1946) reported a lower average age at onset in cases with familial cancer as compared with those having no affected



LEGEND FOR FIGURE 2. Distribution of 201 white breast-cancer patients according to age at onset and total number of pregnancies. The average age at onset for the total group falls within the 5-year interval 50–54 which is blocked off. A line is drawn to connect the average age at onset for each pregnancy group.

relatives. Smithers (1948) did not find that difference between the familial and non-familial cases in his data. The problem requires more study.

Smithers (1948) and also Penrose (1948) discovered that more maternal than paternal relatives were reported to be cancerous. In Penrose's data, 37 maternal relatives and only 18 paternal relatives were reported to have had breast cancer. For other malignancies, 44 maternal and 30 paternal relatives were affected. It was suggested that the difference might be due to a milk agent. One must also recognize that often the woman knows more about her side of the family. In order to have complete records for comparisons, it is necessary to interview the normal relatives on both sides of a family, if possible the female relatives.

Sometimes, reports ignore the possibility that a male without breast cancer may transmit a gene for the tendency. Wood and Darling (1943) reported a family with three sisters having breast cancer. A daughter of one of these also had developed breast cancer. The mother of the three had breast-fed her children, but had not been nursed by her own mother. This was referred to as a possible evidence for milk agent somewhat similar to Bittner's findings with mice. The gene for a tendency could have passed through the non-cancerous mother and maternal grandmother of the three sisters. However, the father of the three was affected and had an affected sister. If heredity is a factor, the gene could have passed through him to his daughters.

Method of Inheritance

Kemp (1948) believes that the various studies on the genetics of cancer support a conclusion that a hereditary predisposition for cancer exists but that other agents may determine localization. Jacobsen (1946) concluded from his study that a hereditary tendency exists for breast cancer and that this tendency has a dominant inheritance. Videbaek (1947) found that the relatives of leukemic patients have a higher incidence of that disease than might be expected by chance alone. He believes that a hereditary predisposition to cancer exists in possibly 20 per cent of persons but that other factors determine the site to be affected.

Penrose (1948b) compared the expected deaths from breast cancer and other milignancies with the observed number among the decreased relatives of breast cancer patients. He found that the number of mothers who died of breast cancer (25) was twice the expected number (11.2). For other malignancies the rates were 51 to 49. There were also 6 mothers alive who had breast cancer and 4 with other cancers. Among the sisters, 23 had died from breast cancer; while 6.98 would be expected to die, on the basis of rate of death from breast cancer in the population. Also ,19 died from cancers of other sites; 25 deaths might be expected. Among the living, 24 sisters had breast cancer and 7 had other sites involved. Because the sisters are more intensely affected than are the mothers, Penrose suggested the possibility that either a recessive inheritance or a fertility factor might be involved. Penrose concluded that a specific factor for mammary cancer and not a general hereditary predisposition to malignancy of any type is transmitted. The method of inheritance was not determinable from his data.

In our studies, the data are too incomplete to permit an analysis for the measure of inheritance or for the method of inheritance. Recognizing that the patients at the interview ordinarily will not be able to give complete data about the family, we work with their living relatives. This is proving to be a fruitful endeavor, as has been explained. The added information about cancer in the family should give data for a more accurate analysis of the inheritance concerned.

Conclusions

Although the heterogeneity of the human population leads to difficulties in the study of genetics of human cancer, the tendency for cancers of specific sites to concentrate in families, where more than one member is cancerous, is suggestive of a hereditary tendency. It is not possible at this time to draw any conclusions as to the method of inheritance. A proper study will necessitate a comparison of the incidences in families of cancer patients with those in a control group. Most of the studies in the past have been weak in this respect.

It is also necessary to collect information about the family from members other than the patients. Economic classes should

not be grouped together for analysis. Our present study includes three racial groups and two economic groups. The data collected about one are as complete as those about the other. The families of the cancer groups, benign breast tumor groups and control groups are followed-up as far as possible. Verifications of all reported cancer among the relatives are attempted. Co-operation from the families, doctors, clinics and hospitals has been gratifying. More study is needed before any conclusions are justifiable. At this time, it is only possible to say that the concentration of sites reported by others is also found in our data.

The study of twins also supports the interpretation that genetics is a factor in cancer occurrence. Monozygotic twins have a high concordance, as compared with dizygotes for both affected, for same site affected, and for age at onset. In studies where data are available, the pathological history also is similar in monozygotic twins.

The majority of the population will be benefited if a genetic tendency for cancer is established. As conditions are now, every person who reaches the age of high cancer risk must pay attention to the possibilities of becoming cancerous. It is doubtful if there are enough facilities available to handle all of the people if the majority begins to seek aid for cancer detection. The increasing age of the population merely adds to the load. The economic factor must also be considered, both the expense and the loss of work hours. Certain privileged few may possibly take advantage of early detection, but for the vast majority of people the problem will be difficult.

If heredity is a factor and a method of inheritance can be determined, the patients and the physician may be helped. Some people can then know that their chance of developing a cancer will be less than that of persons in a "cancer family." Cancer would doubtless develop in some who lack a hereditary susceptibility, particularly if they live under the influence of a strong carcinogen. They present a separate problem.

Other families, because of their histories, would recognize their strong hereditary tendencies to respond to a carcinogenic agent. In fact, only parts of a large family group will have this hereditary background, and they are forewarned. As a conse-

quence, this group should without hesitancy have the regular physical examination. The particular organs or sites which the family history shows to be danger points should be watched carefully. An early diagnosis, made easier by the smaller number of families to watch closely, will save lives. The patient will have the advantage of medical progress in the field during the early stages of the cancer.

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STAGES IN CANCER RESEARCH*

H. P. Rusch

Although the carcinogenic process has never been regarded as a simple one, recent investigations have indicated even greater complexities than were previously suspected. Cancer formation appears not to occur as a continuous single process but rather as a series of biologic changes. Furthermore, there are definite indications that carcinogenic hydrocarbons are active only because of several distinct biologic properties, and neoplasms seem to develop only as the result of the proper sequential summation of these properties. That more than one quality of a carcinogen is involved in the formation of tumors gains support from the fact that some characteristics of hydrocarbons may be masked and others simulated by certain compounds. For example, the observation that an inhibiting effect is exerted on the carcinogenic activity of certain hydrocarbons by wool fat and by liquid petrolatum has been well established (Rusch and Kline, 1946). These anticarcinogens appear to neutralize or to mask the irritating effect of the hydrocarbons without affecting their ability to induce neoplastic cells, since noncarcinogenic chemical or physical irritants quickly call forth neoplasms in tumor-free skin which had previously been treated with the hydrocarbons dissolved in wool fat or liquid petrolatum. When wool fat was used as a solvent for methylcholanthrene, it largely abolished the irritating effects of the hydrocarbon on the skin, and epilation, destruction of sebaceous glands and epidermal hyperplasia were all minimal. Such data suggest at least two biologic properties of carcinogenic hydrocarbons which are essential for the development of tumors: the ability to induce neoplastic cells, and the ability to stimulate these cells to proliferative activity. The factor responsible for the genesis of the tumor is relatively bland and has not been duplicated to our knowledge with noncarcinogenic substitutes, while the property that causes cellular

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multiplication is due to a non-specific irritant of the carcinogen and can be reproduced by other agents.

The present experiments provide further evidence in favor of the concept that cancer formation results from a sequence of biologic changes. As a working hypothesis, the three periods listed in an earlier communication will be considered in light of the more recent findings:

Period of Induction: This stage has also been referred to as the period of preparation, latency, initiation or preneoplasia (Berenblum and Shubik, 1947). Since some arbitrary limitations must be defined, this phase should include only the period of genesis of the neoplastic cell or cells from normal tissue, and it should not be confused with the multiplication of these cells.

Although any concept of carcinogenesis must include a period covering the genesis of the neoplastic cell, the exact sequence of this event in the process has not been demonstrated. Certainly it occurs early, but in certain types of cancer it may be preceded by a period of preneoplasia during which tissues become biased toward forming neoplastic cells. In certain circumstances, such a preliminary phase seems to be essential for the subsequent genesis of tumors. For example, certain cells in atrophied mammary tissue in male mice of high cancer strain may have the potentialities for tumor development, but few if any tumors occur unless the glandular epithelium is first prepared by estrone. Huseby and Bittner (1946) have shown that precancerous nodules appear in mammary tissue prior to cancer formation, and other preneoplastic lesions have been long recognized. It is assumed that the opportunities for neoplastic transformation of normal tissue have been increased considerably during this preliminary period. Thus a "proper soil" appears essential for the genesis of some neoplasms, but there is no reason to believe that such preparation is universally required for all types of tumors. The observation of Nottram (1944) that croton oil accelerates tumor formation when applied to the epidermis prior to the application of a carcinogen is contrary to most findings and must be confirmed, and although Simpson and Cramer (1945) postulate sensitization as a prerequisite to the genesis of the neoplastic cell, our results indicate that stimulation must follow the formation of the

tumor cell. Nevertheless, the available evidence, although not conclusive, is sufficient to justify the subdivision of the period of induction of certain tumors into two phases: (a) stage of preneoplasia and (b) stage of genesis.

Critical Period or Period of Reversibility: This starts at the moment the genesis of the neoplastic cell has been completed, and is that stage in which cellular proliferation is delicately balanced. During this phase there are so few neoplastic cells present that they are more or less lost among the normal cells, and since their mass has not yet attained a size sufficient to receive a direct supply of blood, they must compete with the healthy cells for the nutrients in the fluids of the tissue spaces. At such times these cells are the most susceptible to the influence of their environment; presumably during this phase some tumor cells proliferate, some lie quiescent for varying periods, and others succumb. Small nests of tumor cells have less chance of survival than larger clusters, and the fate of small masses of neoplastic tissue depends on the balance between their proliferative capacity and the amount of encouragement or of resistance present in the neighboring tissues. This period could vary from a few hours to several weeks or months.

The concept of a critical period in the development of a tumor is supported by several types of experimental evidence, but of special consequence is the evidence of a latent period under certain conditions of carcinogenesis. When a carcinogenic hydrocarbon is applied to the skin of mice for a time short of that necessary to induce tumors, the subsequent resumption of the hydrocarbon treatment will quickly precipitate the formation of tumors, even though three to four months intervene between the two periods of application of the hydrocarbon. Actually, in certain cases a noncarcinogenic agent, such as heat, a wound, or croton oil may substitute for the hydrocarbon in the second period (Rusch and Kline, 1946; Berenblum and Shubik, 1947; Friedewald and Rous, 1944). Such observations indicate that the initial application of the hydrocarbon produced alterations in the tissues that persisted for at least four months. We may assume either dormancy of a partially completed chemical reaction which is essential to carcinogenesis and which may be reinitiated and completed by

a second application of the carcinogen at some subsequent date, or we may consider that one phase of the carcinogenic reaction, *i.e.*, the genesis of tumor cells, had been completed and such cells remained quiescent until stimulated to proliferation at some later date. Since the concept of an interrupted chemical reaction does not appear too plausible, it seems more likely that the latter suggestion is the more correct. At all events, it is known that tumor cells may persist in a quiescent state for as long as sixteen to twenty-nine weeks before perceptible growth is observed.

The importance of factors that are responsible for the realization of tumors has been mentioned by several authors, but it is our contention that the influence of these forces is most pronounced during the critical period. Gardner (1939) observed that testicular interstitial cell "tumor rests" could be stimulated to proliferation following the injection of estrogens, and the carcinogenic influence of most chemical and physical irritants is also most effective when applied subsequent to a period of carcinogenic treatment. It is also of interest that high caloric diets have relatively little stimulative effect during the period when cancer is being initiated or on the growth of established tumors, but are definitely stimulative if given just after the preliminary period of carcinogenic treatment, *i.e.*, the critical period (Rusch, Kline and Baumann, 1945).

The length of the critical period, no doubt, varies for different tumors. Neurofibroma developing in the ear of the rat as a result of prolonged feeding of crude ergot regressed when such feeding was discontinued, but recurred when the administration of ergot was resumed. This type of tumor cell failed to proliferate in the absence of certain stimulating factors, and the tumor never became autonomous. The anterior chamber of the eye has been shown to be an excellent location for the growth of many types of tumors, and it illustrates a region of favorable environment for the proliferation of tumor cells during the critical period. Once the neoplasm becomes autonomous, its cells may proliferate in a variety of tissues.

Period of Progression: This may be considered as that period when the neoplastic cells have gained ascendancy over the forces that hold them in control. The tumor has established

its own vascular supply and no longer needs to compete with normal cells for the nutrients in the tissue spaces, but in general is in a period of relatively unrestricted, invasive growth, during which regressions are infrequent. The exact size that a neoplasm must attain before it reaches this stage varies, no doubt, with different tumors, but all grossly perceptible tumors are probably in this phase of their growth. As stated previously, Reinhard, Goltz and Warner (1945) have shown that the chance of survival of a transplantable mouse adenocarcinoma was proportional to the number of cells inoculated and that the latent period for a tumor "take" increased as the number of injected cells decreased. They have further observed more regressing tumors in animals inoculated with dilute suspensions, while none was seen in mice inoculated with concentrated suspensions. If our point of view is correct, it suggests that all inoculated tumor cells, like spontaneous neoplasms, pass through a critical period before they become established. It seems probable that spontaneous tumor cells face a similar critical period—most often, a more precarious one.

The proposed three periods represent only an arbitrary scheme intended to assist in the clarification of our views on carcinogenesis and to facilitate further experimentation. Possibly the different stages are not clearly separable; and within a neoplastic focus at any period, tumor cells are being formed, others are dividing and still others are dying. The final outcome is the result of a balance of the various factors involved. At all events, the ability of tumor cells to remain dormant for long periods and to respond to noncarcinogenic stimulation by multiplying into growth, provides an explanation of those clinical instances in which cancer appears after certain injuries of tissues that had previously seemed normal (Kline and Rusch, 1944; Rusch, 1944; Rusch and Kline, 1948).

Mode of Action of Carcinogens

The finding that certain carcinogens induce mutations has revived the old suggestion that cancer results from a somatic mutation. The induction of mutations in Drosophila with various chemical carcinogens and the finding that the wave lengths that cause mutations in Drosophila (Mackenzie and Muller,

1940) and maize (Stadler and Huber, 1942) are identical with those that cause cancer, are examples of this point. Evidence by Strong (1946) indicates that there is a correlation between the induction of germinal mutations by methylcholanthrene and the unknown changes in somatic tissue which lead to cancer induced by the same compound. An interesting theory concerning the energy relations underlying mutation has been suggested by Schrödinger (1944).

The idea that the significant change in carcinogenesis is an alteration in protein has been suggested previously, but recently Miller and Miller (1947), have observed that a firmly bound compound between proteins of the liver and an azo dye is found in rats fed p-dimethylaminobenzene. It is highly possible that such a combination might initiate the carcinogenic process through sublethal combinations of this dye or its metabolites with critical proteins in normal liver cells and their decendants—the autonomous tumor being the eventual outcome of some of the damaged liver cells through a permanent alteration or loss of proteins essential for the control of growth but not essential for life.

Several attempts to explain the carcinogenic activity of chemicals on the basis of general molecular shape and dimension have been made recently. Others have concerned themselves with correlations of the energy states of the molecule and the carcinogenic activity, but not enough data are available at present to establish firmly such relationships as have been suggested. Several investigators have proposed that it is not the hydrocarbon itself which is the proximate carcinogen, but rather the reactions involved or a possible release of energy, during the transformation from one metabolite to another.

The investigators who have considered a neoplastic cell as a variant or new race of cells have been listed by Haddow (1947), and the most recent suggestion along this line was made by Lederberg (1946) on the basis of studies with mutant strains of Neurospora. Further evidence for a changed race of cells is obtained from experiments in which unicellular organisms were grown in the presence of carcinogens. Under such conditions, a small number of the cells have been shown to undergo an irreversible change and are then able to survive

and multiply in an environment that made this difficult, or even impossible, for their parent cell. The work of Earle (1943) and of Firor and Gey (1945) has shown that normal cells may also be transformed to malignant ones in tissue cultures.

Any complete solution of the mode of action must make clear the relation between physical and chemical carcinogens and the tumor-producing viruses. The possible influence of radiant and chemical carcinogens on certain protein constituents of the cell has been cited, and recently several investigators have discussed the similarity of the viruses to certain constituents in the cytoplasm, *i.e.*, microsomes, plasmagenes, etc.

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PHYSIOLOGICAL EFFECTS OF BETA CHLOROETHYL AMINES OF INTEREST IN THE CHEMOTHERAPY OF NEOPLASMS*

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An interest in the mechanism of action of our tools for the control of neoplastic growth is reflected in the continued study of physiological effects of the mustard compounds. Berenblum, in 1929, reported a series of studies in the anticarcinogenic effects of beta chloroethyl sulfide. This study of the topical use in cutaneous lesions attracted little attention to the potentialities of the problem. The surge of interest accompanied by the effort to develop defensive antidotes and additional tactical agents, revealed the cytotoxic properties upon intravenous administration, which suggested the present clinical application (Goodman, 1946; Jacobson, 1946; and Wintrobe 1946). The first interest in the topical therapeutic use of the compounds to be fund in the literature is a report of Adair and Bagg in 1931.

We are familiar with the syndrome of nausea and vomiting of central origin, which develops following intravenous injection of these compounds. The delayed toxicity manifested by atrophy of the lymphatic system and the bone marrow and intestinal epithelium results from the proclivity of the compound for rapidly proliferating cells.

Above the LD_{50} dose certain characteristic pharmacologic actions are manifested on the central nervous system. These are a cholinergic action which is possessed by both the mustard and the nitrogen mustard compounds, and is characteristized by a muscarinic action on the glands and the smooth muscles. In both of these agents, peripheral stimulation appears to account for the muscarinic response, although central excitation cannot be excluded (Graef, *et al.*, 1948).

What is the mechanism which produced these changes? Is it possible that the adrenal may be an intermediary? Is this a result of stimulus by some noxious agent or a basic chemical

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interference with the normal physiologic process of the individual cells?

Ludewig and Chanutin (1946) have considered the possibility that these changes may be mediated through the adrenal in a manner first described by Selye who stressed the effect of noxious stimuli upon the adrenal, producing a hypertrophy of the cortex. On this basis, they have studied the adrenal before and after the injection of mustard compounds and have seen that there is a marked hypertrophy of the cortex. This enlargement is due chiefly to an increased water content and, to some extent, to proteins and phospholipids. The total cholesterol concentration of the adrenals is markedly decreased due to loss of cholesterol esters. The total lipid concentrations decrease. These changes are not dependent on variations in either the cholesterol or the phospholipid concentrations. It may be said then that the changes seen in the adrenals of these animals is the end result of a non-specific stimulation of the "alarm reaction" type which is entering a phase of exhaustion.

White and Dougherty (1944) found that the total serum protein increased slightly, soon after the injection of adrenocorticotrophic hormone in rats. They related this change to the destruction of lymphocytes following the injection. These workers showed that both the beta and gamma globulin increased in normal rabbits during the first day following injection of either adrenocorticotrophic hormone or adrenal-cortical steroids. These and other findings have been interpreted to mean that the lymphoid tissue is a source of the globulin. Chanutin and Gjessing (1946) and Gjessing and Chanutin (1946) have studied further the possibility that a similar effect may be produced in the animals subjected to nitrogen mustard. By means of electrophoretic analysis they have shown that there is a latent period of two to three days before changes in the electrophoretic pattern become definite. There is a rather marked change in the alpha globulin, which in a large part may be due to several new protein fractions, one of which is extremely rich in lipids and another which is poor in lipids. There is also a characteristic increase in beta globulin which he believes to be due to the addition of new proteins to the plasma. These findings also suggest that the changes seen in

nitrogen mustard intoxication are not mediated through the adrenal.

Kornofsky, *et al.* (1948), have undertaken more acute studies in which they have observed the manifestations of intoxication in adrenalectomized and normal animals. They conclude that the "alarm reaction" must be considered in interpreting the toxic effect of any drug, but that it plays only a minor role in the production of the characteristic pattern of injury seen in nitrogen mustard intoxication. They concur with earlier workers that the toxic effect of this compound is very rapidly expressed upon the individual cells. This was demonstrated by means of occluding the circulation of a small portion of the intentine for five to fifteen minutes after the injection of a lethal dose of nitrogen mustard. The protected portion was spared the damaging effects of the compound. By occluding circulation of the lower extremity with a clamp on the abdominal aorta and inferior vena cava for two to fifteen minutes after injection of nitrogen mustard, granulocytopenia in the peripheral blood was prevented; and, at autopsy, hyperplasia of the femoral marrow was noted, while aplasia was present in the other marrow areas.

Can the observation on single cells, then, help us in an understanding of the physiological effects of nitrogen mustard? The studies of Gillette and Bodenstein (1946) on the embryonic amphibian development are of particular interest in this respect. They describe a multitude of effects upon various developing tissues of the embryo which are extremely interesting, but these cannot be discussed in detail at this time. Of particular interest to us is the cessation of mitosis in the epidermal cells with nuclei developing to a stage of interphase and remaining so throughout the period of observation. Nuclear enlargement can be seen to progress to a maximal stage, after which the fragmentation appears. This breakdown affects only the nuclei, not the cytoplasm. During the period of development, however, the cell becomes abnormally large and there is a fragmentation of basophilic nuclei. It is of interest that Painter and Taylor (1942) and others, have found that in such growing cells ribose nucleic acid is synthesized. One might believe, then, that in such exposed cells an abundant

synthesis of ribose nucleic acid has occurred, but that mustard has interfered with the mechanism which transforms the ribose nucleic acid into the desoxyribose type of chromatin.

Fell and Allsop (1948) have studied the influence of mustard gas upon the living cells *in vitro* using small pieces of choroid and sclera from ten- to twelve-day chick embryos. An attempt was made not only to observe morphological changes, but also to follow histochemical developments. Their observation showed that cytological effects of mustard gas result in the rapid coagulation or fixation of the cell with little distortion of their shape and remarkably good preservation of the nuclear chromatin. This preservation of the chromatin structures, which also characterizes the central areas of mustard gas lesions in the human and animal skin, can be achieved by rapid and complete precipitation of nucleo-proteins, as had been previously described by Berenblum (1947). This fixation effect does not prevent mustard gas from infiltrating into the cells and accumulating in the fat vacuoles. By the use of a selenium dioxide reagent it has been shown that mustard is present in these vacuoles. In dilute concentrations, mustard gas does not produce this coagulation of protoplasma, but it seems to reduce its viscosity and render the chromatin structures almost unrecognizable. This type of damage might well be caused by an interference with the enzyme systems. These workers also noted many abnormal mitotic figures in cultures grown in sublethal concentrations of mustard; in fact, they comment specifically on the similarity of these changes to those described by Strangeways in tissue cultures subjected to X-ray irradiation. It should be mentioned that severe mitotic disturbances resulting from mustard have been described by Koller (1947) in Tradescantia. Auerbach and Robson (1947) have seen mutations induced by exposure of Drosophila to a suitable concentration of mustard. The findings of Gillette and Bodenstein (1946) reveal that there is a selectivity of this substance in various tissues and that mitosis, and not differentiation, has been strikingly inhibited. They illustrate examples of epidermal cells in which the two processes are present in the same cell. Mitosis stopped but the cells continued to grow, and thus became abnormally enlarged. In the large cells, characteristic histological differentiation appeared at the same time

as it did in the more numerous and smaller cells of the unexposed animal.

If, then, this change may be related to nucleic acid, determinations of the nature of such inhibition are in order. Bodenstein and Kondritzer (1946) have determined chemically the ribonucleic acid and desoxyribo-nucleic acid content of a series of successively older normal amphibian embryos and embryos treated with nitrogen mustard. The analyses showed that during normal development of the stages investigated, the content of both nucleic acids rises steadily as the age of the embryo increases, and reaches its peak at the time that all of the yolk is absorbed. In the exposed embryos, on the other hand, only the desoxy-ribo-nucleic acid remained at the level reached at the time of exposure. The nitrogen mustard treatment thus prevented the synthesis of desoxy-ribonucleic acid in the embryos. Since we know little of the metabolism of nucleic acid we cannot as yet explain this in terms of the effect of nitrogen mustard in body chemical systems.

Again, it is of interest to point out the similarity between certain mustard and X-ray effects. Mitchell, using Casperson's method, showed that X-ray blocked the synthesis of desoxyribose nucleic acid. X-ray and ultraviolet irradiation also reduced the viscosity of desoxyribose nucleic acid by the depolymerization of the long molecules. This has been shown by Taylor and others (1947). Gjessing and Chanutin (1946) have shown that nitrogen mustard compounds will depolymerize sodium thymonucleates. These workers have also shown that this effect may be neutralized by sodium thiosulfate which reacts with the chloroethyl group, preventing the formation of the imino rings degradation compounds. This ability of the nitrogen mustards to depolymerize thymonucleate has also been demonstrated by the use of ultraviolet absorption spectrum.

The group at St. Bartholmew's working under the direction of Wormall (Boursnell, *et al.*, 1946, a, b, c, d), have attempted to elucidate the mechanism of the action of the mustard compounds (dichlorodiethyl sulfide) on protein. Early experiments by this group revealed that the mustard reacted rather promptly and strongly with amino acids abolishing, in particu-

lar, the free sulfhydryl groups. This property was present in the beta chloroethyl diethyl sulfone and the divinyl sulfones. In order to gain more precise information as to the reaction in the complicated protein molecule and the distribution of the material in the tissues of the body, preparations containing radioactive sulfur and deuterium were prepared. It became apparent that there was a fixation of the mustard by human serum proteins in a ratio of about 22 moles per 16 gams of protein nitrogen. There was a marked reduction of free sulfhydryl, and the amount of sulfur entering the serum proteins may be ten times the equivalent of titrable sulfhydryl groups. Blocking of the free amino groups with phenyl-isocyanate does not reduce the capacity of the proteins to react with nitrogen mustard. However, the degradation products of mustard appear to react readily with the amino groups (Banks, *et al.*, 1946).

A very interesting group of experiments was undertaken to determine the distribution of mustard gas following intravenous injection. The distribution of radioactive sulfur in the tissues of rabbits after injection of five mg/kg of mustard showed greatest localization to be in the kidney, lung and liver, organs which may serve excretory functions. The small amount in the bone marrow may be significant in view of the high reactivity of this tissue. Three main conclusions may be reached from this experiment. First, most of the injected mustard or hydrolysis products rapidly diffuse from the blood stream. Second, there is a widespread distribution of sulfur in the tissues of the injected animals, with retention of the fixed sulfur in these tissues for some considerable time after injection. However, there does not appear to be any obvious relationship between the fixed sulfur and the extent of damage occurring in any particular tissue. Third, there is a rapid excretion (20 minutes), starting shortly after the injection of radioactive sulfur-containing compound. When the various hydrolysis products of mustard gas are injected in a form containing the radioactive sulfur, a similar distribution of material was noted.

An extensive study of the effect of nitrogen mustard on enzymes and tissue metabolism was undertaken by Barron

and his group (1948, a, b,) as well as by the Cori's (1948). Golumbic, Bergman and Fruton (1946) demonstrated that tertiiary halogenated alkylamines, when in aqueous neutral solutions, undergo a series of rapid transformations, the first of which is formation of an ethylenimonium ring. This processs proceeds very rapidly, while the further degradation is at least 100 times slower. The resemblance of these transformation products to choline is striking. The similarity between ethylenimonium derivatives of nitrogen mustard and choline, and acetylcholine, suggests the possibility that they may act as structural inhibitors of enzymes by combination with the side chains of the protein moiety where combination of choline or acetyl-choline takes place. The validity of this assumption was demonstrated by experiments with choline oxidase, acetylcholine esterase and choline acetylase. By a series of studies of choline oxidase inhibitions, it became apparent that the formation of ethylenimonium rings was essential to this inhibition. One important difference between the nitrogen mustard inhibition and other substrate inhibitions is that it is irreversible and it does not depend upon the ratio of the substrate to the inhibitor. It became of interest, then, to explore the possible means of inhibiting this reaction of choline oxidase. The substances of interest include thiosulfate, BAL (British-Anti-lewisite), urotropin, glutathione, and thiourea. A concentration of thiosulfate a thousand times greater than the concentration of nitrogen mustard prevents completely the inhibition of choline oxidase by nitrogen mustard. A significant depression of the effect of nitrogen mustard on the enzyme was not obtained by the other substances.

They also react with a large number of organic compounds of biological importance, including the amino groups, peptides, proteins, nicotinic acid, thiamine, adenylic acid, adenosinetriphosphate, pyridoxine and organic phosphorus compounds. Some of these are essential for enzyme activity. This high reactivity of halogenated alkylamines would suggest that they are powerful inhibitors of a large number of enzymes. However, experiments performed by a number of investigators, including Peters, Dixon and Cori, do not support this contention. In fact, if *in vitro* experiments with concentrations above 10

times the lethal dose, or about 1 x 10^{-4} M are discarded, the only enzyme systems inhibited are choline oxidase, choline esterase and pyruvate oxidase. Cori, *et al.* (1948), have studied in detail the effect of nitrogen mustard (MBZ) on the enzyme system dealing with phosphorus exchange. They found, in certain tissues of animals injected with methylbis-beta-choloroethyl amine or the ethylenimine transformation product, a partial inhibition of inorganic pyrophosphatase, phosphocreatine phosphokinase, and hexokinase. It may, therefore, be concluded that these three phosphorus exchange enzymes are inhibited by nitrogen mustard at concentrations around the lethal dose.

In tissue metabolism studies it has been illustrated by Barron, *et al.* (1948), that the pyruvate metabolism and the oxidation of 1-amino acids are inhibited. This may be due to the combination with the sulfhydryl groups of the protein moiety of these enzymes, or to a combination of other groups of the side chain of the protein with the highly reactive halogen groups of nitrogen mustard. The lack of inhibition of succinoxidase and phosphoglyceraldehyde dehydrogenase is the only evidence that nitrogen mustards do not inhibit all sulfhydryl enzymes. In fact, inhibition of mitosis by nitrogen mustards, sulfur mustards and X-ray, finds reasonable explanation in the assumption that mitosis is controlled by sulfhydryl-containing enzymes easily aylkylated or oxidized.

The great sensitivity of bone marrow respiration explains the leukotoxic action of nitrogen mustard. The inhibitory activity of mustard can be reduced by the addition of choline or choline precursors or dimethylethylamino-ethanol, or methionine; and illustrates the role of choline in metabolism of bone marrow. This work may, however, point a way to treatment of mustard toxicity or to the development of a selective action of these compounds in the protection of specific tissues. Thus, if protection of the hemopoietic system is achieved, a greater concentration of mustard may be administered to individuals with neoplasms. This has not been successful to date.

Another inviting approach to the development of more effective chemotherapeutic agents is a selective inhibition on nucleoprotein metabolism. Attention has been directed to the inhibitory effects of amino-stilbenes by Kopáč (1947) and others.

Under the direction of Dr. Alexander Haddow (1948), a group at the Royal Cancer Hospital has been studying the 4-aminostilbene and have considered the possibility of altering its properties by varying the basic side chains. One compound of interest is 4-N, N-di-(2 chloroethyl)-aminostilbene. Administration of this substance in relatively high doses, about 500 mg per kg to rats bearing the Walker carcinoma, produced an inhibition of tumor growth of considerable degree but of brief duration. Pharmacologically, the compound appeared to be different from the aliphatic chloroethyl amines on one hand, and the aminostilbenes on the other. Extensive studies have been carried out to explore the properties of other aromatic compounds bearing aliphatic amine side chains (the N-N-di-(2 chloroethyl) derivatives of aniline, o-, m-, and p-toluline, o- and p-anisidine, beta, naphthylamine, and p-amino-benzoric acid) which would exhibit specific cytotoxic and inhibitory activity as judged from the growth of various animal tumors. The toluidine and naphthylamine compounds showed perceptible advantages because of their reduced toxicity. The administration of single intraperitoneal injections of 70 to 100 mg/kg in a solution of arachis oil on the day following implantation with the Walker tumor resulted in a considerable increase in the survival period and inhibited the development of the graft completely in about one half the animals. Similar results were also obtained when these compounds were administered orally. While less marked results have been obtained in the cases of various mouse neoplasms, including spontaneous mammary cancer, Crocker sarcoma 180, and transplantable sarcoma, an outstanding inhibition of lymphosarcoma, spontaneous and transmitted leukemia originating in Furth AK-1, pure line, is expressed in much longer survival than can be achieved in the same tumor with urethane. Further work must be continued to determine the optimal structural requirements for biological activity. However, the increased inhibitory activity of these compounds with lower toxicity is fruitful evidence of a pursuit for some substance which may offer more prolonged and perhaps more selective inhibition in nucleoprotein metabolism.

SUMMARY

In reviewing the physiological effects of the mustard compounds in terms of chemical alterations, it has been demonstrated that such alterations are remarkably similar to those produced by X-ray. In our expanding knowledge of the enzyme systems involved in the process of growth, it is possible that a method for selective inhibition of growth processes may be developed.

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THE USE OF YOLK SAC CULTIVATED TUMORS IN CANCER CHEMOTHERAPY STUDIES*

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Cancer chemotherapy is still very much in the pioneer stage. Leads are few and uncertain. A large number of compounds must be tested in the hope of discovering specific antagonists to neoplastic tissue. Since the possible substances are practically unlimited and since a particular compound must usually be assayed several times under varying conditions of dosage and manner of administration before its value can be decided, it is most desirable to be able to run preliminary or screening tests as rapidly as possible.

For several years at The University of Texas Biochemical Institute, we have been exploring the possibility of using yolk sac cultivated tumors in cancer chemotherapy studies. Our experience with yolk sac cultures of tumor tissue has been extensive. This method of growing tumors in embryonated eggs was originated at the Biochemical Institute in 1941 and has been used in association with various research projects since that time. One strain of mouse mammary carcinoma has been cultivated continuously by serial transplantation in eggs for more than one hundred sixty transplant generations or nearly seven years.

The first attempts to use yolk sac cultivated tumors for testing the possible anticancer effect of certain chemicals was undertaken about five years ago. At that time, however, there were too many variables still uncontrolled to make the project of practical value. Since then the yolk sac technique has been very much improved and better methods for introducing the test compound into the egg have been developed (Taylor and Carmichael, 1948). Yolk sac tumors can now be produced which grow as rapidly and as uniformly as transplants of the same tumor in its natural host (Figure 1).

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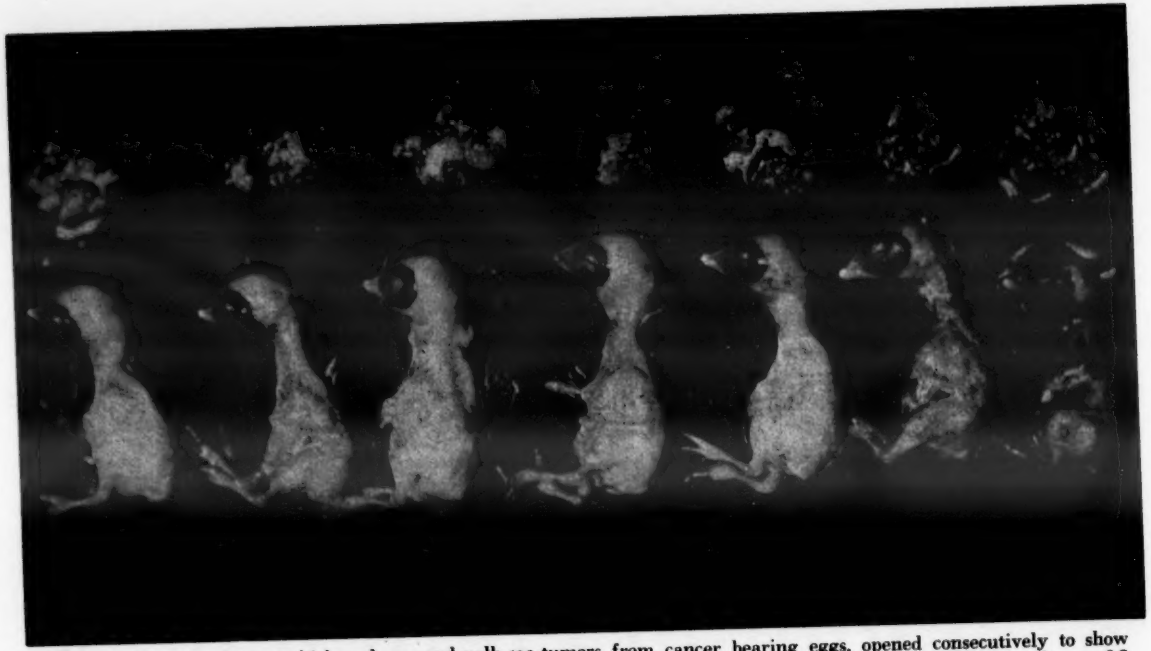

FIG. 1. A series of seven chick embryos and yolk sac tumors from cancer bearing eggs, opened consecutively to show size and variation on the 14th day of incubation. Average tumor size for the series, $1.4 \mp .3$ gms., chick embryos, 6.2 $\pm$ .5 gms.

During the past year, egg-grown tumors have been used to test the anti-cancer possibilities of numerous materials. The method has been found to be especially useful for preliminary evaluation of compounds. Parallel with these studies, the same compounds have been tested on fast growing mouse transplants. It has been found that where dosage and other factors are comparable, tumor tissue reacts similarly, whether in the mouse or in the egg.

The use of yolk sac tumors in cancer chemotherapy enables the investigator to screen through compounds more quickly and with much less overhead of work and materials. Further, the laboratory time given to these studies can be quickly expanded or contracted as the need arises. Perhaps the main advantage of the egg-grown tumor over the mouse transplant is the presence in the egg of fast growing non-tumor tissue along with the tumor. Such data as are available indicate embryo tissue to be biochemically and physiologically nearer to tumor tissue than any other non-tumor tissue. Hence, the reaction of the check embryo serves as a rather delicate index of the specificity of any effects the introduced material may have on the yolk sac tumor.

Materials and Methods

Yolk sac tumors. A C3H mouse mammary carcinoma which originated spontaneously in breeder stock was used in these studies. It was carried for several transplant generations in mice after which it was inoculated into eggs by the yolk sac technique (Taylor, Carmichael, and Norris, 1948). It proved to be exceptionally adapted to propagation in the yolk sac and regularly attained a size of 1.0 to 1.5 gm. ten days after yolk sac inoculation. The growth rate in its natural host was much less.

The method of yolk sac inoculation was essentially as previously reported (Taylor, Carmichael, and Norris, 1948). A few minor modifications have been introduced. The tumor tissue was injected into the yolk sac suspended in 0.85% saline instead of the mixture of egg white and saline used before. It has also been found preferable to use one part tumor to eight parts saline rather than the 1:4 dilution used previously, and to inject 0.4 cc. of the suspension into each egg. The same

syringe and needle may be used for the injection of ten or twelve eggs, if before each injection the needle is dipped into alcohol and flamed. Experiments have demonstrated the importance of keeping the eggs as near as possible at their incubation temperature during the process of inoculation with tumor tissue. When the eggs are allowed to remain for long periods at room temperature the yolk sac tumors are much reduced in size. The eggs for tumor inoculation should be removed from the incubator in small batches and immediately returned after the operation is completed so that there is only a short exposure to room temperature.

It has been definitely established that the continued passage of a tumor through the yolk sac tends to adversely affect the chick embryo, resulting in smaller yolk sac tumors (Taylor and Carmichael, 1949). This transfer toxic effect begins to appear in the tumor used in these experiments at about the fourth to sixth serial transplantation in the yolk sac. One or more transplantations back into the normal host, the C3H mouse, eliminated the toxic effect for another four to six yolk sac transplant generations.

The yolk sac tumor used in these studies averaged 0.3 gm., eight days after tumor tissue was injected into the eggs, or after twelve days of total egg incubation. On the 13th, 14th, and 15th day of incubation the tumor size averaged 0.6, 1.2, and 1.9 gm. respectively. The tumor increases in size 400% in 48 hours between the 12th and the 14th day of incubation. Figure 2 illustrates the growth of both embryo and tumor during this stage of egg incubation. This period of rapid growth has proved to be especially suitable for cancer chemotherapy tests. Either a 24 or 48 hour interval may be used. Most of the experiments reported here utilized the 48 hour time interval.

Cancer chemotherapy tests. Tumor-bearing eggs on the twelfth day of incubation were allowed to remain in one position for several hours or over night, so that the embryo and chorio-allantoic membranes were located in the dorsal area of the egg. The eggs were divided into control and experimental groups (18 to 20 eggs per group). Care was taken to select the eggs so that such factors as egg size and previous treatment were the same for each group. Then by use of an egg candler, a

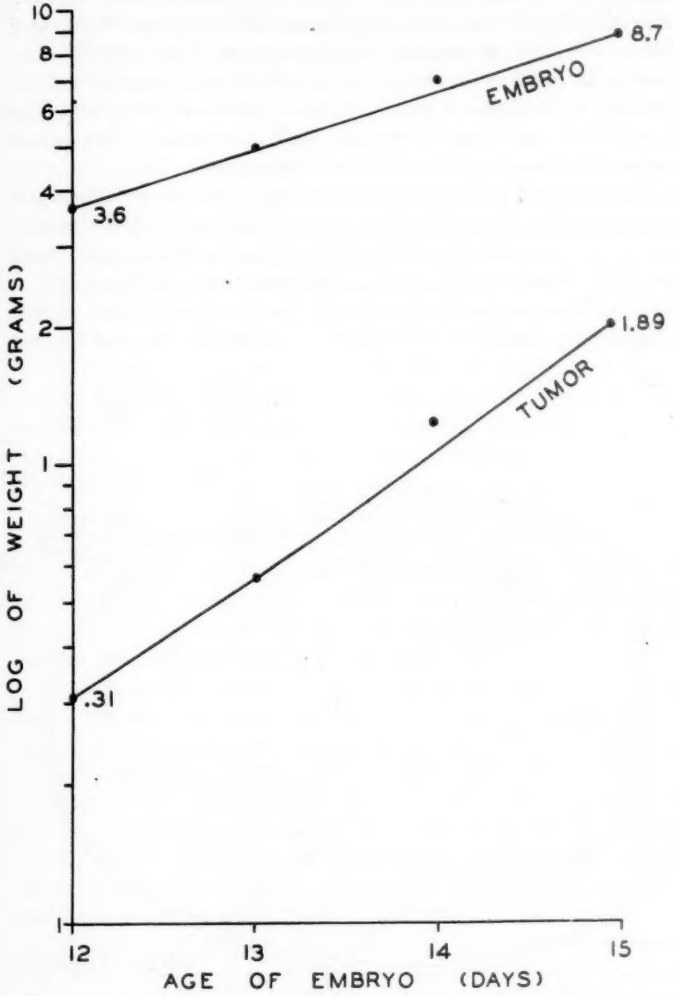

FIG. 2. Growth rate of chick embryo and yolk sac tumor from the 12th to the 15th day of incubation.

small circle about one-fourth inch in diameter was marked over the blood vessels of the chorio-allantoic membrane well below the embryo amniotic sac and large blood vessels. The shell area within the circle was scrubbed with slightly iodized alcohol, then dented with a moderately blunt scissor point, care being taken not to perforate the shell membrane. The scissor point was flamed before each denting operation.

The material to be tested was injected with a 1 cc. tuberculin syringe and a 22 or 23 gauge needle. The point of the needle, bevel up, was inserted at the dented area so that it penetrated the shell membrane at a slant, depositing the inoculum, 0.1 to 0.2 cc., between the shell and the chorio-allantoic membranes (Figure 3). For injection into the yolk sac, the needle was

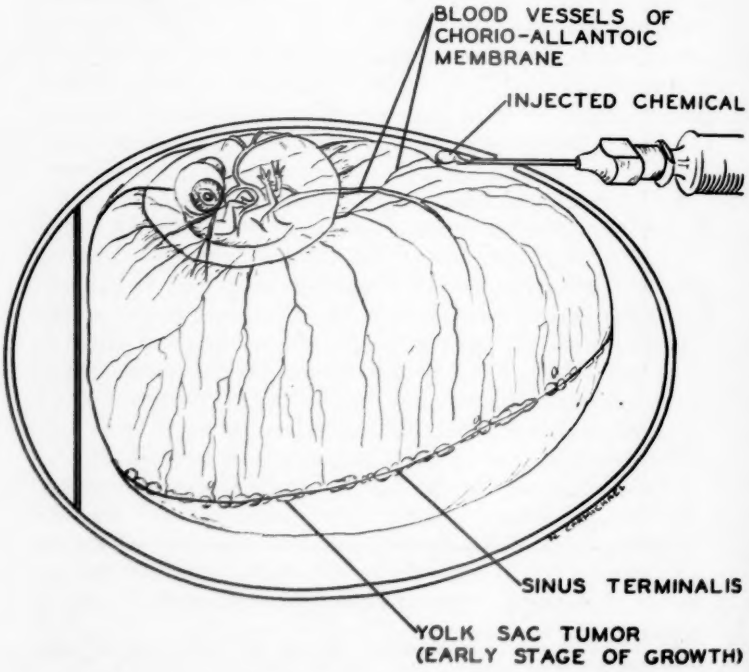

FIG. 3. Twelve-day-old tumor bearing egg showing needle in position for injection of materials between the shell and chorio-allantoic membranes.

slanted toward the center of the egg and inserted about one-half to one inch. When done carefully and the needle withdrawn cautiously there was no regurgitation of fluids injected in this manner, as the shell membrane formed a seal around the needle. The needle was dipped into alcohol and flamed before each injection. The procedure caused no injury, and eggs so treated with 0.2 cc. of saline or other non-toxic material showed the same survival and embryo growth rate as untreated controls. After inoculation, the shell puncture was swabbed with iodized alcohol and sealed with paraffin.

Experimental and control eggs were incubated at 37.2°C. It is important to treat the eggs alike with respect to temperature. Most incubators do not maintain a uniform temperature in all parts, so that it is necessary to mix the experimental and control eggs on each shelf used. Care must also be taken not to leave the eggs at room temperature any longer than necessary, and especially to guard against a differential in this respect between the experimental and control groups.

The eggs of an experiment, in most instances, received a single injection, but it has been found that each egg will tolerate at least four injections over the chorio-allantoic membranes of 0.2 cc. each at intervals of four hours or more. The procedure for the second, third and fourth injections was the same as that outlined for the first inoculation.

Various types of suspending media have been used. Tests with dyes have demonstrated that the excretory system of the twelve-day embryo quickly eliminates into the allantoic fluid material injected over the chorio-allantoic membrane. A saline solution of non-toxic compounds was completely excreted in less than twelve hours. Hence, in order to maintain the test material at a relatively high level in the blood of the embryo for longer periods of time, suspensions or solutions in a non-toxic oil were necessary.

Cottonseed oil proved to be inocuous when introduced over the chick blood vessels or into the yolk sac. In these experiments, the compounds tested were, when possible, first dissolved in 0.85% saline and then an emulsion of one part saline to two parts cottonseed oil used as the suspending media for the egg injections.

When compounds were introduced into the yolk sac they were taken up into the chick embryo blood system at a slower and more uniform rate. Since, however, the yolk material contains so many unknowns chemically, it appeared that in most instances the action of the introduced chemical could be best evaluated when deposited directly over the chick blood vessels as in the membrane technique.

The tumor bearing eggs after injection with the test compound were usually incubated for 48 hours before harvesting tumors and embryos for evaluation of the results of the experiment. In some instances the eggs were incubated for 24 hours. The longer period allows for much more tumor growth, and if the chemical used has any inhibitory effect it will be more definitely demonstrated.

Tumor and embryo of each egg were weighed and examined for significant gross or microscopic morphological changes. When the average weights of the tumors or embryos differed in the experimental as compared with the control group, the experiment was repeated and the data subjected to statistical analysis in order to determine the validity of the difference. The death rate in the experimental and control eggs was also recorded.

Results

Table I shows the results obtained with para-aminobenzoic acid. This vitamin at high levels, 1 mg. or more per egg, consistently inhibited tumor growth 20 to 40% during the 48 hour test period. Inspection of this table indicates that, using yolk sac tumors, only 60 tumor bearing eggs and three experiments were necessary to obtain statistically valid data on a growth differential between the control and experimental eggs of 20%. The chick embryo in this experiment was unaffected.

Table II lists the results obtained with high levels of eight other B vitamins. There was no satistically valid difference in tumor or embryo size between experimental and control series.

Many other chemicals have been tested. During the past year, 47 compounds or materials of unknown chemical structure have been screened by the egg technique. This has involved 238 experiments and a total of 7,066 tumor bearing eggs. Detailed reports on some of these will be given later.

TABLE 1

The effect of high levels of para-aminobenzoic acid on the growth of yolk sac cultivated mouse mammary carcinoma. 48 hour test.

Dosage (mg.)	No. experiments	No. eggs tested	No. eggs harvested	Average tumor size (gm.) Control	Exp.	S. D. Control	Exp.	Experimental tumor size (% of control)	Statistical validity difference / P. E. of difference
1	3	60	50	1.31	1.04	.551	.508	79.4	3.0
3	3	62	57	1.33	.83	.586	.427	62.4	5.9

TABLE 2

The effect of high levels of B vitamins on the growth of yolk sac cultivated mouse mammary carcinoma. 48 hour tests.

Material	Dosage (mg.)	No. of experiments	No. eggs tested	No. eggs harvested	Average tumor size (gm.) Control	Exp.	Average chick size (g.m.) Control	Exp.	Experimental tumor size (% of control)
Biotin	3–10	6	131	111	1.18	1.03	6.6	6.5	87.3
Folic acid	3– 5	5	92	80	1.36	1.21	6.7	6.7	89.0
Inositol	2–10	3	63	54	1.16	1.00	7.0	6.7	86.0
Niacin	2–10	3	56	47	1.32	1.32	6.9	6.6	100.0
Pantothenate ..	2–10	3	60	48	1.31	1.28	6.4	6.4	97.7
Pyridoxine	3–14	6	114	92	1.32	1.20	6.5	6.4	90.9
Riboflavin	2– 3	3	56	51	1.26	.97	6.5	6.5	77.0
Thiamine	3–10	4	78	68	1.37	1.12	6.7	6.5	81.8

Conclusion

Yolk sac cultivated tumor tissue has been found to have certain advantages for the evaluation of possible anti-cancer compounds. These may be summarized as follows:

1. The growth rate of the tumor and the embryo is such that 24 to 48 hour tests are practical.
2. Tumor and chick embryo share a common blood stream in the egg so that the reaction of both cancer and rapidly growing non-cancer tissue to the test compound can be measured.
3. This method of screening possible anti-cancer compounds requires much less work and is more economical than the use of mouse grown tumors.

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GENETOTROPHIC DISEASES; ALCOHOLISM*

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If attention is centered on the classification of those diseases in which no infective agent appears to be involved, *nutritional* diseases such as pallagra, rickets, scurvy and beri beri, and diseases of *genetic* origin such as hemophilia, Huntington's chorea, etc., constitute highly important groups.

So far as we know, however, no one has given attention to the existence of *genetotrophic* diseases—those in which the genetic background and nutritional factors jointly enter into the etiology. Recent developments in the field of biochemical genetics (1) make it inescapable that each individual possesses a distinctive hereditary make-up and a metabolism which is distinctive as to its details because of differences in enzymic patterns (2, 3). Partial genetic blocks (4, 5) somewhere along the metabolic assembly line are probably common place in the inheritance of individuals and those may give rise to augmented requirements for specific minerals, vitamins, amino acids or other nutritional factors. On the basis of these genetic variations it becomes possible for one individual to suffer from nutritional disease when his diet is wholly adequate from the standpoint of many other individuals.

We offer the hypothesis that numerous diseases of obscure etiology are genetotrophic in origin. With respect to alcoholism, we have strong evidence which is to be presented in this paper. Other diseases such as allergies, mental diseases, cardiovascular diseases, arthritis, multiple sclerosis, drug addition and cancer have not been studied from this standpoint, but we believe that the facts warrant such a study and that careful consideration should be given to the possibility and even probability in some cases that genetotrophic factors are operative.

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*From the Biochemical Institute and the Chemistry Department of The University of Texas and the Clayton Foundation for Research, Austin. This research was supported in part by grants from the Research Corporation and the Research Council on Problems of Alcohol, New York. Received for publication January 9, 1950.

That these factors enter in an important way into the etiology of a particular disease does not exclude the entrance also of psychogenic or other influences. Even diseases which are primarily due to infective agents may in turn be greatly affected by genetotrophic factors. An individual may, for example, possess a metabolic pattern which is conducive to a specific nutritional deficiency; this deficiency in turn may constitute an invitation to infection even though the available evidence on this point is not impressive (6).

Alcoholism and Metabolic Patterns

Alcoholism or compulsive drinking considered as a disease continues to be one for which neither a practical means of prevention nor a satisfactory treatment have been developed (7).

One of us, on the basis of new clarification and insights gained in the field of biochemical genetics and on a careful survey of the pertinent literature, has outlined reasons for suspecting that the extreme appetite for alcohol possessed by compulsive drinkers has a physiological basis closely linked with the inherited metabolic patterns of the individuals afflicted (8). Other specific appetites, *e.g.*, for salt, for calcium, for phospate, for sugar as in certain diabetics, for fat, for protein, for B vitamins, all have a physiological basis and there is ample reason for supposing that the appetite for alcohol has also.

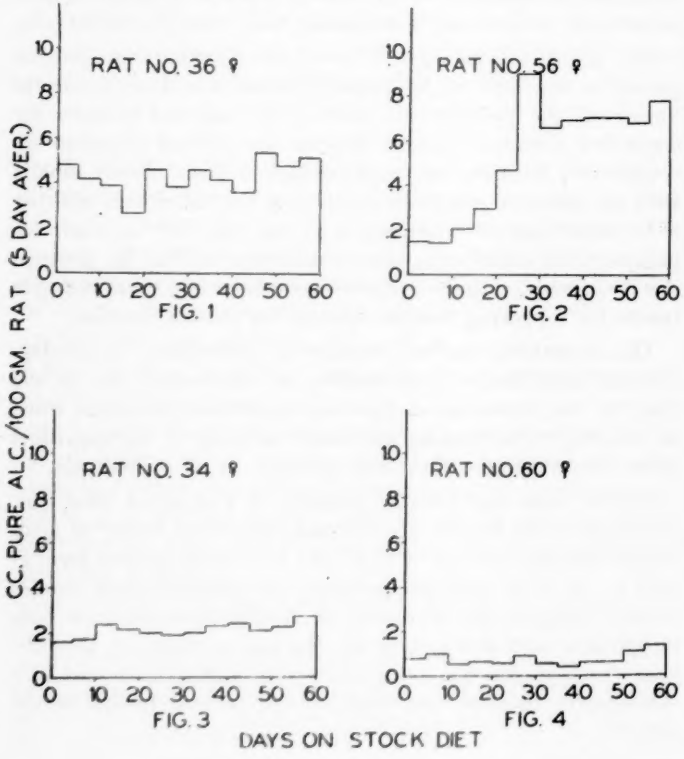
This hypothesis does not exclude the influence of psychological and social forces in alcoholism but emphasizes that in the study of the physiological aspects of alcoholism attention must be directed to individual metabolic patterns as distinguished from the hypothetical pattern common to all individuals.

Studies with experimental animals here reported offer confirmation of this hypothesis and make possible a theory of alcoholism which is in line with all the previously known facts as well as the new findings presented. In addition, these studies strongly suggest the possibility of developing treatments both prophylatic and therapeutic for the management of this disease. Such treatments will, in the nature of the case, act in a constructive manner and improve the general health of the patient.

Individual Patterns in Rats and Mice

On the basis of the study of the individual alcohol consumption curves of over 100 rats and about 30 mice, it is clear that each animal tends to exhibit a distinctive pattern of response to alcohol. These patterns, however, fall into a few general groups, and show a parallel to the diverse drinking patterns of human individuals. For these experiments the animals were kept in individual cages with access *ad libitum* to supplies of both 10 per cent alcohol and water, and the positions of the two drinking bottles were interchanged daily.

One group of rats, on a stock diet, exemplified by Figure I, consumed relatively large amounts of alcohol rather consis-



tently, often beginning the first day. Others (Figure II) avoided the consumption of alcohol at first but after a time drank more and more until after a few weeks a large part of their fluid intake was 10 per cent alcohol. Still others (Figures III and IV) avoided the consumption of substantial amounts of alchohol over a period of many weeks. A closer examination of the consumption curves shows them to be highly individual in character. In some animals the consumption was relatively steady, in others it tended to fluctuate widely. In Figure III it will be noted that the consumption was consistently higher than that shown in Figure IV but continued for a long period at a very moderate level. Further evidence as to the distinctive character of the individual responses will be presented later.

Effect of Diet in Experiments with Groups of Animals

When groups of animals were placed upon experimental instead of stock diets, the average responses were found to be dependent upon the type of diet used, in confirmation of the earlier work of Mardones, Segovia and Onfray (9) and Brady and Westerfeld (10). These workers have found that groups of rats on restricted diets consume larger amounts of alcohol than when the restricted diets are supplemented with B vitamins and liver and yeast extracts. Mardones, Segovia and Onfray have postulated the existence of an unknown principle, "Factor N," which controls appetite for alcohol. Brady and Westerfeld also observed the efficacy of a liver extract preparation in decreasing alcohol consumption under certain conditions, but after the alcohol consumption became relatively large they were unable to observe more than a temporary diminution of consumption caused by its addition to the diet.

The two experimental diets used principally in these experiments were designated Diet A and Diet B. Diet A contained

	Parts	
Commercial casein	20	
Sucrose	60	
Vegetable oil (Wesson Oil)	15	(fortified to give 60 units
Dried brewers' yeast (autoclaved)	10	Vitamin A and 10 units
Salt mixture No. 2	5	Vitamin D per cc.)

The yeast was autoclaved at pH 9.0 for 90 minutes at 125° C.

Diet B had the same composition except for the addition of

	mg. per kgm. of diet
Thiamine	1
Pyridoxine	1.5
Riboflavin	7.5
Choline	1000
Calcium Pantothenate	30
Inositol	30
p-Aminobenzoic Acid	30
Folic Acid (synthetic *L. casei* factor)	1
Nicotinic Acid	10
Biotin	0.1

In Tables I and II are included summaries of the data on alcohol consumption with respect to two strains of rats on Diet A and Diet B. "Strain O" is used to designate the original stock of Wistar rats which had been grown in our laboratory for a period of years and "Strain H" to designate similar rats obtained from a neighboring laboratory.

TABLE I

Alcohol Consumption of Rats and Mice

Type Animal Studied	Number of Animals	Diet*	Av. Alcohol Consumption 25–40th Day as cm. 3/100 gms. Animal/Day	Range of Alcohol Consumption	Mean of Deviation from Mean	Percentage of Deviation from Mean
Strain "O" Rats	10	A	.93	.48–1.39	.21	23%
Strain "O" Rats	10	B	.17	.07–0.29	.05	30%
Strain "H" Rats	41	A	.73	.13–1.83	.25	34%
Strain "H" Rats	38	B	.27	.07–0.63	.11	41%
dba Strain Mice	10	A	.29†	.24–0.38	.036	12%
C_3H Strain Mice	9	A	.46†	.07–1.38	.42	91%
White Mice	14	A	.50†	.23–1.87	.27	54%

*The rats were on the diet and had access to alcohol 25 days prior to the period covered in this table, and the mice were on the regimen for 5 days.

†The period for the mice was from the 6th to the 14th day.

All animals were started on diets within one week from weaning.

TABLE II

Comparison of Two Strains with Respect to Their Maximum Alcohol Consumption

Strain	Number of Rats	Diet	Average Maximum Alcohol Consumption During Period on Diet	Range	Average No. Days to Reach Maximum	Range of Days Individual Rats)
"O"	26	A	.94 ± .24	.58–1.54	53	35–81
"H"	39	A	1.33 ± .35	.72–2.17	52	35–81
"O"	26	B	.35 ± .14	.08–0.75	81	62–120
"H"	42	B	.47 ± .23	.03–1.00	61	53–87

It will be noted that Diet A induces far greater alcohol consumption than Diet B and that the maximum consumption (Table II) is reached earlier on Diet A than on Diet B.

Genetic Basis for Alcohol Response Patterns

It has previously been shown that ordinary inbred strains of laboratory animals are by no means homogeneous with respect to their nutritional requirements and that substrains of such animals with different levels of requirement for specific vitamins can be bred (11, 12). From the data presented in Table 1 several pertinent observations may be made:

(1) While "Strain O" and "Strain H" rats both consume much more alcohol on Diet A than on Diet B, the contrast between the two diets is significantly greater in the case of "Strain O" rats.

(2) On Diet A "Strain O" rats consumed 27 per cent more alcohol than did "Strain H" rats.

(3) On Diet B, however, the situation was reversed; the "Strain H" rats consumed 60 per cent more alcohol.

(4) Variability within the group is relatively high in the case of the two strains of rats in the C_3H mice and the white mice, whereas the variability is relatively low among the dba mice.

These observations find a ready explanation on the basis of genetic differences, and no other explanation has suggested itself. In the case of the dba mice which are being used extensively in our laboratories in cancer investigations, brother-sister matings have been strictly adhered to and this appears to account for the low variability in this group. The C_3H

mice ordinarily are handled in the same manner, but breeding difficulties have been encountered and brother-sister matings had to be abandoned until the difficulties could be eliminated. The two strains of rats and white mice are of the sort commonly used for nutritional investigations and no special precautions have been taken in connection with their breeding.

It should be noted that the extreme variability exhibited by the C_3H mice is due in part to the fact that for these animals Diet A is very deficient. The experiment involving these mice could not have been extended much longer because of inadequacy of the diet. Strain differences in nutritional requirements of this sort have doubtless a genetic origin.

The existence of wide variations in individual responses (Tables I and II), which, however, are minimized in the dba mice, can hardly be explained on a genetic basis, and the high variability with respect to treatment to be discussed later offers further strong evidence as to the importance of genetic factors in determining the responses to alcohol.

Control of Alcohol Appetite in Animals

Perhaps the most striking evidence of genetic variability was obtained in connection with the treatments which were instituted to overcome the alcohol appetite of the rats. Animals of "Strain O" and of "Strain H," for example, responded in a significantly different manner when they were administered 10 B vitamins by mouth and the anti-pernicious anemia vitamin by injection. In every one (100%) of the 24 rats of "Strain O," the alcohol consumption was decreased to a low level by this treatment. In 70% of these rats no difficulty was encountered in keeping the alcohol consumption at a low level. In the other 30%, there was a tendency to revert to a higher level as in the work of Brady and Westerfeld, which tendency, however, in our experiments could usually be overcome by repeated vitamin administration.

Among the 45 rats of "Strain H" treated in the same manner, only 3, or about 7%, showed a diminution of alcohol consumption to a low level. In view of the relatively large number of animals used and the consistency of the results, there seems no

question as to the significance of the observed differences between the two strains.

A large number of rats have been treated in the course of our investigation. Typical results can best be presented in the form of a series of individual consumption curves on which are indicated in code the treatments that were instituted. These results show that for individual rats on the indicated diets different nutritional factors enter into the creation of an appetite for alcohol. For example, one rat "Strain O" (Figure 5) was dramatically and permanently cured of its desire for alcohol by the oral administration of 10 known B vitamins alone. At the other extreme is Rat No. 1, "Strain H" (Figure 13), the alcohol consumption of which remained at a high level even after 3 other vitamins had been administered, viz., Vitamins A, E and the anti-pernicious anemia vitamin. When some linseed oil was added to the diet, however, the alcohol consumption promptly fell to nearly zero. Various intermediate variations are depicted in Figures 6 to 12. Figure 6 represents the consumption curve of a rat which was very slow in developing an appetite which, however, was dramatically abolished by the administration of 10 known B vitamins plus the anti-pernicious anemia vitamin. Figure 7 has to do with a rat which drank steadily over a long period at a low level. Its appetite too was abolished by a similar vitamin treatment. Another rat (Figure 8) remained at a high level of intake for nearly seven weeks, when the administration of 11 vitamins promptly brought the consumption down to nearly zero. Rat No. 70 (Figure 9) is one for which the anti-pernicious anemia vitamin appears to be unusually important. This is somewhat true of Rat No. 69 (Figure 10) which showed an initial response to the anti-pernicious anemia vitamin alone, showed an increase when the B vitamins were first administered, but subsequently a drop to approximately zero when a second treatment with the anti-pernicious anemia vitamin was given. Figure 11 depicts the case of a rat of "Strain H" which had its appetite greatly decreased by the administration of 11 vitamins. This rat had access to alcohol for 55 days previous to the treatment, but it consumption record for the earlier period was not taken.

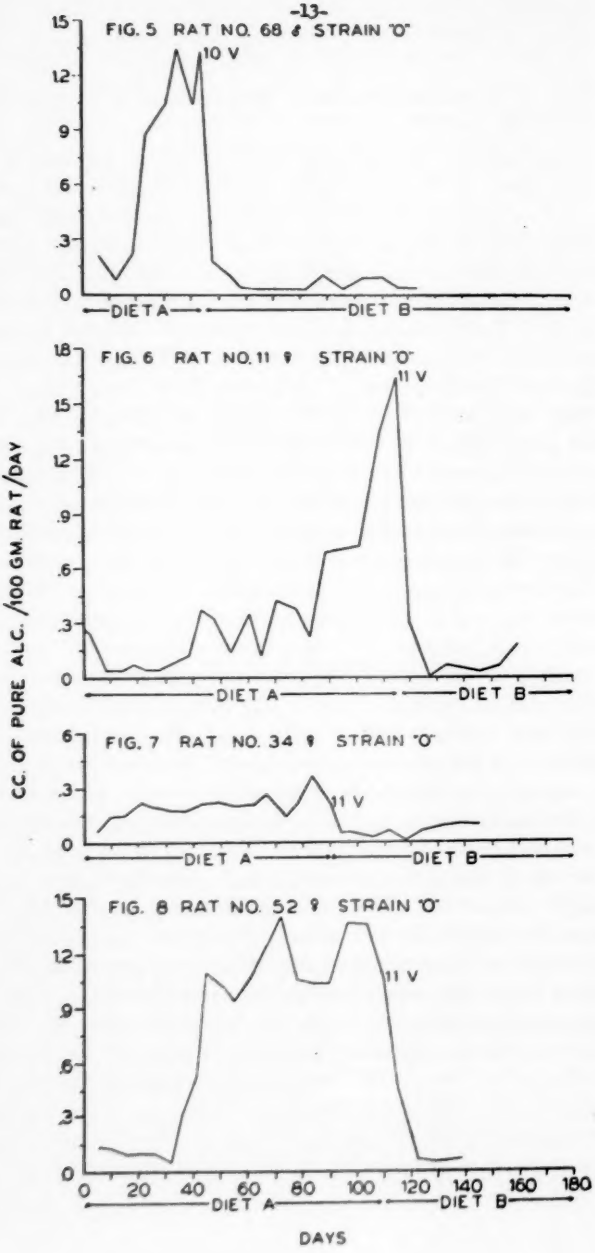


FIG. 5 RAT NO. 68 ♂ STRAIN "O"
10 V
DIET A
DIET B
FIG. 6 RAT NO. 11 ♀ STRAIN "O"
11 V
DIET A
DIET B
FIG. 7 RAT NO. 34 ♀ STRAIN "O"
11 V
DIET A
DIET B
FIG. 8 RAT NO. 52 ♀ STRAIN "O"
11 V
DIET A
DIET B
CC. OF PURE ALC. /100 GM. RAT/DAY
DAYS

Code for Alcohol Consumption Curves

1V

B_{12} — 5 units of 20 unit anti-pernicious anemia liver preparation given intraperitoneally each day for 5 consecutive days.

4V

	Per Day for 5 Days
Thiamine	0.3 mg.
Riboflavin	0.5 mg.
Calcium Pantothenate	3.0 mg.
Folic Acid (Foluite)	0.1 mg.

5V

Same as 1V + 4V, except that the B_{12} was partially purified.

10V

Same as 4V with the addition of

	Per Day for 5 Days
Pyridoxine	0.15 mg.
Choline	100.0 mg.
Inositol	3.0 mg.
p-Aminobenzoic Acid	3.0 mg.
Nicotinic Acid	1.0 mg.
Biotin	0.01 mg.

11V

Same as 1V + 10V.

13V

Same as 11V with the addition of

	Per Day for 5 Days
Vitamin A	500 units
Vitamin D	100 units

L

Same as 13V with the addition of

Linseed oil ..... 5% by weight to total diet

NOTE: In the alcohol consumption curves the experimentally determined values are plotted. These are uncorrected for spillage and evaporation. Hence, a low value may actually represent zero consumption.

In Figures 12 and 13 it will be noted that additional Vitamin A administration appeared to be effective in bringing about a material diminution of alcohol consumption in "Strain H" rats. A diminution was observed in two days in every one, 100% of the 31 rats furnished additional Vitamin A along with the other 12 vitamin supplements already furnished. In another series of experiments the addition of linseed oil to the diet caused a marked diminution of the alcohol consumption in four out of six rats. Two such cases are depicted in Figures 12 and 13.

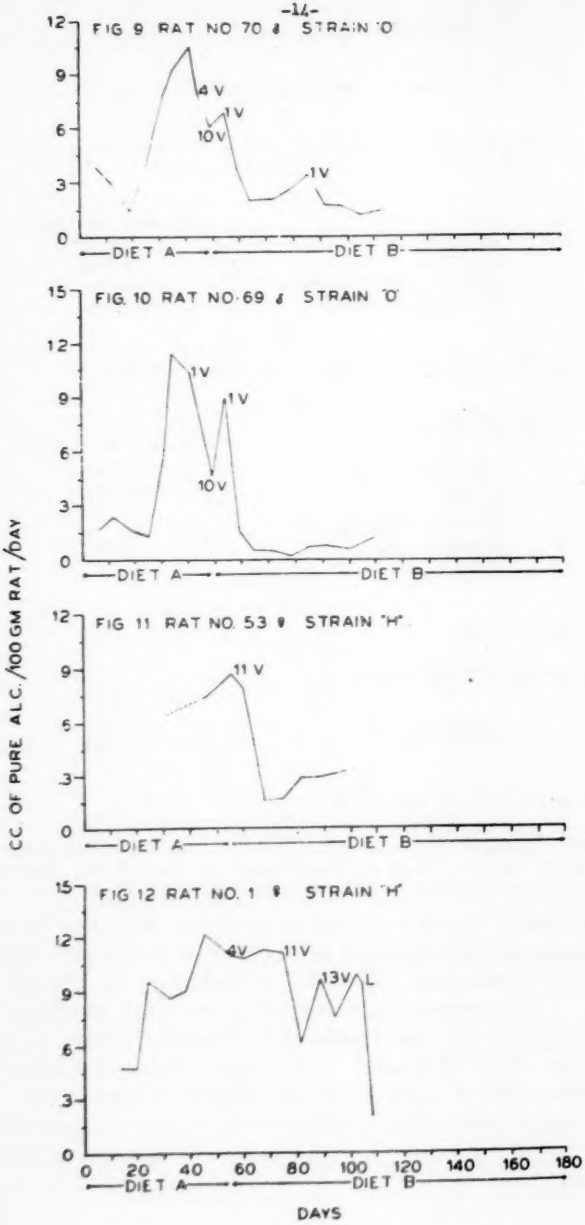
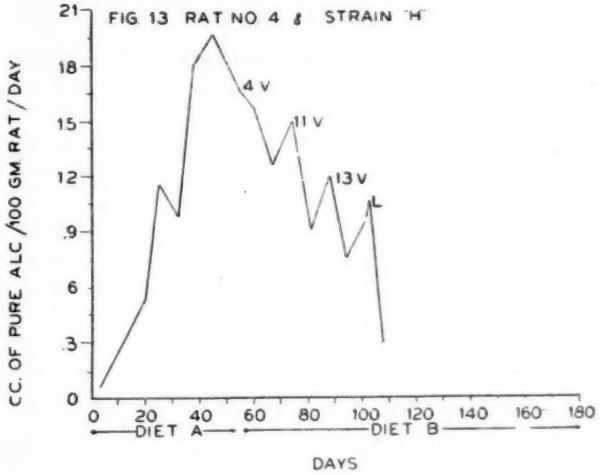


FIG 9 RAT NO 70 ♂ STRAIN 'O'
4 V
1 V
10 V
1 V
DIET A
DIET B
FIG. 10 RAT NO 69 ♂ STRAIN 'O'
1 V
1 V
10 V
DIET A
DIET B
FIG 11 RAT NO. 53 ♀ STRAIN 'H'
11 V
DIET A
DIET B
FIG 12 RAT NO. 1 ♀ STRAIN 'H'
4V
11 V
13V
L
DIET A
DIET B
CC. OF PURE ALC. /100 GM RAT /DAY
DAYS



Our experimentation in the control of alcohol appetite in mice has not been extensive. The experiments which have been performed indicate that the problem with mice is not essentially different than with rats. Only six mice have been treated and these for only four days at the date of this writing; of these, five diminished their intake, one remained unchanged. The average diminution for the six was 54%. One had diminished its consumption to zero.

It is clear from our experience that extended studies must be made before it will be possible to abolish with complete success the alcohol appetite of every individual laboratory animal regardless of its peculiar inheritance. We have not yet investigated in the general field of amino acids or minerals; it may be that in some animals a partial genetic block causes a high requirement for one of these and that such deficiency induces in some unknown manner an abnormal appetite for alcohol. Our experience convinces us, however, that it will be possible by an extension of the means we have already employed to abolish the appetite for alcohol in 100 per cent of laboratory animals.

Habituation Experiments

It has been tacitly assumed in connection with a number of studies (13) that animals can be habituated to alcohol simply by forcing them to drink it over a period of time. According to the genetotrophic concept this should not be the case for all the animals if the diet of the animals is a reasonably satisfactory one.

While our experiments on this subject have not been carried very far, certainly they indicate strongly that habituation, particularly in the sense of addiction, is not brought about by the simple procedure that has had wide use.

A group of 9 weanling rats was placed for 29 days on Diet B and furnished as the only available fluid 10 per cent alcohol. A similar group of rats was placed on the same regimen except that a stock diet was used instead of Diet B. Two control groups (one on each diet) were placed upon the same regimen except that they were given a choice from the start between 10 per cent alcohol and water.

At the end of the 29–day period the four groups all had a choice between water and 10 per cent alcohol as in the earlier experiments described. The results presented in Table III

TABLE III

Effect of Forced Alcohol Consumption ("Habituation") on Voluntary Intake of Alcohol

Group	No. of Rats	Diet	cc. Alcohol per 100 gms. Rat per Day	Range	Mean of Deviation from Mean	Percentage of Deviation from Mean
"Habituated"	9	B	.09	.06–.13	± .01	11%
"Habituated"	8	Stock	.06	.05–.09	± .01	17%
Control	18	B	.15	.07–.25	± .05	33%
Control	8	Stock	.32	.07–.78	± .18	51%

show that animals which are forced to take 10 per cent alcohol for a period of time are by no means led to take large amounts by choice. There is the strong suggestion that when rats on essentially adequate diets are forced to drink alcohol, they actually develop a dislike for it and drink less than if they had been given a choice from the beginning. In any event, this procedure which has often been used in animal studies on alcohol-

ism is clearly not promising as a means of producing compulsive drinkers.

Theory of Alcoholism

The theory suggested and supported by these experimental findings may be outlined as follows: Appetite for alcohol is a physiological perversion based upon incompletely satisfied nutritional needs. We know relatively little as to the mechanism whereby physiological changes in the body induce special appetites, but that special appetites are induced by the internal environment is undeniable. Alcoholic craving according to our theory develops as an overpowering drive in certain individuals as a result of their unusually high requirement for one or more specific nutritional entities, such as the B vitamins, and because this high need is not fully satisfied by the common foods which are consumed. The use of large quantities of refined foods would, according to this theory, contribute to alcoholism; and the consumption of alcohol itself, in addition to developing a taste, would promote the appetite and start a vicious cycle because it crowds out of the diet wholesome foods which normally contribute to the various needs.

There may or may not exist a few specific lacks (known and unknown) which are generally associated with the compulsive drinker's appetite. Our experiments with rats indicate that widely different deficiencies may contribute directly or indirectly in producing an appetite for alcohol. Among the controlling factors are various of the known B vitamins, the anti-pernicious anemia vitamin (vitamin B_{12}), Vitamin A, and unsaturated fat acids (linseed oil). Our knowledge of precisely how the anti-pernicious anemia vitamin is obtained and assimilated from natural diets is limited. It was administered to the animals by intraperitoneal injection in order to insure its absorption. The failure to assimilate this or some other vitamin might lie at the basis of certain cases of alcoholism.

Our experimental studies strongly suggest that the *abundant* satisfaction of *every* nutritional need will abolish alcoholic appetite. It should be emphasized that in providing for every nutritional need the chain is as strong as its weakest link and satisfying every need except one should be expected to be com-

pletely ineffective. Furthermore, due partly to differences in assimilation, bacterial action in the intestines, etc., as well as distinctive features in the pattern of intermediate metabolism, the requirement for a given vitamin or other nutritional entity by a particular individual may actually be far above what is considered normal. This will make it more difficult than might be supposed to insure to him an adequate supply of every potential nutritional factor.

It is true that the treatment of alcoholism has often included the administration of supposedly generous amounts of B vitamins. A sufficient cause for the failure of vitamin therapy as a treatment for alcoholism in the past is the omission of the anti-pernicious anemia vitamin as well as sufficient amounts of certain other vitamins which in individual cases may be necessary links in the chain. Commercial vitamin preparations arae often inadequate and poorly balanced (14), and the idea back of whatever therapy that has been used has been to remedy deficiencies arising because of excessive alcoholic consumption. No one, so far as we know, has ever seriously considered a nutritional deficiency to be a cause of alcoholism, and no attempts have been made therefore to correct an underlying deficiency. Requirements in such cases may be very high or hard to satisfy, and haphazard administration of "vitamin pills" and self-medication by uninformed laymen can be expected to be completely ineffective or even harmful or dangerous.

The effects of very high alcoholic consumption have not as yet been studied in our investigation. On a body-weight basis the maximum consumption observed under our conditions is equivalent to 3.7 qt. of 90 proof liquor per day for an average man. In proportion to the total food consumption of a rat, however, the amounts are much smaller. It may be that continued nutritional deprivation associated with high alcoholic consumption does irreparable damage to the individual and that for this reason advanced stages of alcoholism will be incurable by satisfying all the nutritional needs. We may hope that is not so, and the presumptive evidence is against this idea.

The theory which we have set forth, if valid, emphasizes the importance of good nutrition and the desirability of fulfilling

the nutritional needs of everyone—not only those who have average requirements. In calculating nutritional needs, a far larger factor of safety than is generally used may well be in order. It seems, on the basis of our experiments, very likely indeed that consistent good nutrition from childhood on will strongly militate against alcoholism. Regardless of the efficacy of any treatment which has its basis in the theory propounded, the prophylactic effects of good nutrition are strongly indicated. Good nutrition may, however, mean in some cases nutrition designed for the needs of a particular individual with specific requirements that are unusually high.

An interesting manner in which the proposed theory fits the facts has to do with the relationships between age and alcoholism. If an individual becomes an alcoholic at an early age, before 28 (15), up to now there has been practically no hope for him. In general, those who become alcoholic later in life are much more susceptible to treatment. According to our theory, those individuals who become alcoholics early are those who have the highest requirement for some specific nutritional factor; the resulting deficiency obviously shows up earlier and the possibility of its being met or even halfway met is remote. Those who become alcoholic in later years do not have such marked deficiencies and the urge can perhaps be broken by psychological means.

Need for Further Research

Study of alcoholism from the point of view we have developed is in its infancy, and much further research will be required to clarify the problem and to test various ramifications of the theory.

One line of research must center on the problem of precisely what nutritional deficiencies may be responsible for inducing an appetite for alcohol. We hope to contribute materially to the available information in this field, but we also invite the participation of other investigators.

Another research field of large proportions has to do with developing means of assessing individual metabolic patterns, so that something other than a "shotgun" therapy can be used. We believe that it will eventually become possible through the

efforts of many investigators in many laboratories to determine in the case of any specific alcoholic the biochemical roots of his particular difficulty and on this basis to formulate an adequate treatment.

It is obvious that a large amount of clinical investigation will be required before satisfactory human treatments can be evolved based upon our animal experiments. Clinical trials are being instituted in various localities, and it is hoped that all clinicians who study the problem will give the broad theory underlying our observations intelligent and careful study and trial.

In connection with clinical studies, a few comments may properly be made. Because alcoholism appears to arise because of different deficiencies in different individuals, it will be necessary for the physician to exercise judgment in the handling of each individual case. No perfectly uniform regimen is likely to be effective. It should be remembered, however, that different nutritional factors are often interdependent, and therefore it is essential that *every nutritional need be met simultaneously.*

It should be noted also that even if one has relieved the appetite of an alcoholic, no lasting benefit would result were he allowed to go his way, become deficient again and relapse into the condition of alcohol craving again. How much danger will exist in this direction, only experience can show. It is obvious, however, that a one-time alcoholic should continue to have his nutritional needs satisfied continuously throughout life.

A decidedly hopeful aspect of the problem is the fact that many alcoholics have a genuine desire to overcome their difficulty. The indefinable appetite, which is comparable to the craving of some diabetics for sugar or a drug addict for his drug, is too strong to be overcome by psychological means. It seems most probable on the basis of our laboratory experiments that fully satisfying the nutritional needs will at least take the edge off the appetite so that the alcoholic can be rehabilitated and will not be forced to use more will power than he possesses. In our experiments with rats, which admittedly may not be classified as addicts, we are able to abolish their drinking practically entirely even though alcohol is immediately available

to them continuously without their expending any effort to obtain it. Because of the desire of most alcoholics to avoid alcoholic consumption, this demonstration with rats constitutes a much more severe test than would be encountered in human situations.

Finally, another vast field of research which is opened up by the theory we have proposed is the possible relation of genetotrophic factors to other diseases of obscure etiology, which have previously been mentioned.

We gratefully acknowledge our indebtedness to our colleagues and assistants, to Helen Kirby, who has carried forward the investigation of individual metabolic traits, and to Janet Reed, Nellie Keffer, William Brown and Gene Rich, whose technical assistance has been invaluable.

Summary

Evidence is presented based upon animal experimentation that alcoholism is a genetotropic disease, viz., a disease in which genetic factors and nutritional deficiency together are etiological agents. It has been found possible by the administration of nutritional factors to control the appetite for alcohol in laboratory animals. It is suggested that genetotrophic factors may be important in many diseases of obscure etiology.

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LACK OF ANTAGONISM OF MALONONITRILE AND DESOXYRIBONUCLEIC ACID TO TRYPANOSOMIASIS, ALLOXAN DIABETES AND LETHAL EFFECTS OF X-IRRADIATION*

JAMES PRESTON PARKER, PAUL L. EWING AND G. A. EMERSON

Loevenhart *et al.* (1918) studied effects of cyanide on respiration and reported evidence of cerebral stimulation in certain mental diseases. Marshall and Rosenfeld (1937) found that pyruvic acid cyanohydrin produces prolonged stimulation of respiration through a cyanide effect, and Heymans and Masoin (1897) related the toxic effects of malononitrile to cyanide poisoning. Hydén and Hartelius (1948) demonstrated an increased amount of nucleoprotein in the cytoplasm of anterior horn cells, spinal ganglion cells and ganglion cells of the frontal cortex of rabbits treated with 4 mg./kg. of malononitrile, but not in cells of the parotid salivary gland, liver or pancreas. Szanto and Felsenfeld (1949) attributed the prophylactic and curative effects of malononitrile on poliomyelitis in mice to a change in intracellular environment in which nucleic acids of the cells are unavailable for nutritional demands of the virus, and Hydén and Hartelius (1948) concluded that beneficial results of malononitrile therapy in mental diseases were due to increased nucleoprotein content of cytoplasm of the neurons.

It was of interest to test the effects of an agent powerfully influencing ribonucleotide metabolism on three conditions in which equilibrium of this metabolism may be considered to be markedly disturbed. X-irradiation causes nuclear damage and significant changes in tinctorial properties of cells, particularly of the gastroenteric tract; ribonucleotides and dimercaptopropanol have no significant prophylactic effect on these changes in rats (Thurlo B. Thomas, personal communication), although cysteine was found by Patt *et al.* (1949), to increase survival of rats exposed to 800 *r*. Alloxan diabetes is com-

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pletely prevented by administration of ribonucleotides prior to alloxan, in much smaller doses than those of equivalently prophylactic sulfhydril compounds (Emerson *et al.*, 1946). *Trypanosoma equiperdum* infection, which is fulminant in rats and mice, presumably involves a significant transfer of nucleotides from host to parasite, although survival in this infection is not appreciably altered by ribonucleotides (Ewing *et al.* (1950), or by stimulation of the reticuloendothelial system (Ewing *et al.*, 1946). An extension of our observations of effects of desoxyribonucleic acid in these three conditions was also considered desirable, particularly with massive doses of this agent. The present study describes tests indicating the lack of effect of large doses of malononitrile and desoxyribonucleic acid on any of these three conditions.

Experimental

A generous supply of ampules containing 700 mg. of sterile malononitrile was furnished by Dr. Edward Henderson, Schering Corporation, Bloomfield, N. J. This material was shipped in solid CO_2, and was kept in the laboratory at approximately 4°C. For injection, 0.03% saline solutions were prepared at room temperature and used immediately. Occasionally, these solutions were cooled to 4°C. and used within 5 hours after preparation. All injections of malononitrile were made intraperitoneally.

Desoxyribonucleic acid was obtained from Schwarz Laboratories, Inc., New York. Small amounts of 7% aqueous solutions were made at a time, buffered with $NaHCO_3$, and used immediately. Solutions appeared grossly to become more viscous on standing at 4°C. All injections of this agent were made intramuscularly.

Experimental Trypanosomiasis. A standard intraperitoneal inoculum of *T. equiperdum* obtained by diluting blood of an infected mouse with saline has been found to kill within 96–120 hours. Fifteen adult white mice were so infected and divided into 3 groups of 5 mice each. To the first group, 8 injections of 3 mg./kg. of malononitrile were given intraperitoneally at 52, 57, 60, 72, 78, 82, 85 and 100 hours after inoculation. The second group received 700 mg./kg. of desoxyribonucleic acid at each of these intervals. The third group received no

treatment. The total dose of malononitrile given in 48 hours was 24 mg./kg., and of desoxyribonucleic acid, 5.6 g./kg. None of the mice died from acute effects of these agents. Frequent blood examinations were made, and trypanosome counts were repeatedly made after 72 hours of infection. There was no significant difference in the rates of increase in parasitemia on any of the 3 groups. The mean times of death for the three groups were 113±12, 120±14 and 108±2.5 hours, respectively.

Feulgen stains of blood smears taken at 85 and 100 hours from all the mice, and a few taken terminally, showed no regular differences in tinctorial properties of leucocytes, although there may have been a tendency toward deeper staining and leucocytosis in the group treated with desoxyribonucleic acid. Trypanosomes were not sufficiently well stained, after a 20-minute hydrolysis, to permit comparisons between groups.

Alloxan Diabetes. Fourteen white mice, divided into 3 groups, were injected intraperitoneally with 200 mg./kg. of alloxan monohydrate after a 20-hour fast. The first group, comprising 4 mice, was treated with 3 doses of 3 mg./kg. of malononitrile, 20, 15 and 1 hours before alloxan was given; an additional mouse succumbed to the third injection, and all showed accentuated stimulant effects of malononitrile after fasting. The fast was continued for 2 hours after injection of alloxan. Malononitrile injections were given 4, 9, 28, 33, 51, 56, 75 and 80 hours after alloxan was administered. The second group was similarly treated with 700 mg./kg. of desoxyribonucleic acid at the same intervals before and after alloxan. The third group received no treatment except alloxan. Total doses given were 33 mg./kg. of malononitrile and 7.7 g./kg. of desoxyribonucleic acid, over 4 days. All the mice with the exception of one in the untreated group became severely diabetic, with >500 mg. % of reducing substances in the blood; the remaining mouse did not have >180 mg. % at any time during the period of observation, and its values are omitted from the mean values below.

Blood content of reducing substances was estimated by a modification of the method of Somogyi (1945), using 0.02 cc. of blood from the tail. Mean fasting blood values were 48, 96, and 53 mg. %, respectively, for the three groups; the high value

for the group treated with desoxyribonucleic acid may be due to the large dose of this agent given 1 hour before testing. The mean values at various intervals after injection of alloxan were: at 1 hour, with the mice still fasting, 104, 97, 70; at 25 hours, 501, 615, 499; at 51 hours, 851, 917, 627; at 73 hours, 842, 948, 612; at 96 hours, 743, 902, 568; and at 117 hours, 649, 587, and 577 mg. % for the three groups, respectively. One of the 4 mice treated with malononitrile died in 7 days after alloxan, and another during the second week. All of the mice treated with desoxyribonucleic acid died within 6 days. Of the controls, two died within 4 days and another in 10 days. Blood values for the 2 surviving mice which received malononitrile were 492 and 460, and for the surviving diabetic control, 401 mg. % on the 14th day after alloxan.

Lethal Effects of X-Irradiation. Twelve mice were exposed to 900 *r* of X-irradiation, with radiation factors of 200 kv., 18 milliamps., 0.5 mm. Cu and 0.5 mm. Al filters, and a target distance of 40 cm. Half of the mice were exposed at a time, and the 12 were distributed equally in 3 groups of 4 mice each in regard to the two exposures. Mice in the first group received 3 mg./kg. of malononitrile 28, 23, 8 and 4 hours prior to exposure and 1, 21, 26, 42, 47, 66, and 72 hours after exposure. Those of the second group received 700 mg./kg. of desoxyribonucleic acid at the same intervals. The third group received no treatment. The mean survival times were 6.75 ± 3.0, 4.75 ± 0.5 and 6.75 ± 2.7 days, respectively, with ranges of 4–11, 4–5, and 4–10 days for the three groups.

Discussion

The above results indicate, despite the small series of observations, that there is no effective antagonism by malononitrile or desoxyribonucleic acid of the 3 conditions noted, since massive repeated doses of these agents were given. The question may be raised as to the restriction of the effects of malononitrile to the central nervous system. Hydén and Hartelius (1948) suggest that the effect of malononitrile is to increase production of ribonucleotides by an action on enzymatic mechanisms, which is most probable since the amounts given are inadequate to serve as substrate in any direct reaction. It is difficult to conceive of restriction of these enzyme effects

to neurons alone, without assuming either a marked neurotropy for malononitrile or a specific sensitivity of enzymatic mechanisms in the nervous system. The first assumption is improbable for a compound with a low partition coefficient, and the second would present an unusual property of enzymes. Whatever the effect of malononitrile on ribonucleotide metabolism, the disturbance is not beneficial in the conditions studied.

Lack of effect of desoxyribonucleic acid confirms our previous findings in its action in alloxan diabetes, and is probably related to different metabolic pathways for ribo- and desoxyribonucleic acids.

SUMMARY

Neither malononitrile nor desoxyribonucleic acid, in massive repeated doses, has appreciable effects on experimental trypanosomiasis, alloxan diabetes, or lethal effects of X-irradiation in mice.

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A MEASURE OF INTRARENAL PRESSURE*

A. V. Montgomery, E. R. Mickle, H. G. Swann, and J. L. Coleman

The importance of intrarenal pressure as a factor in renal physiology and pathology has been stressed by isolated investigators since the close of the 19th century. Harrison (1896) reported a difference in "kidney tension" or hardness concomitant with various types of nephritis. He stated that with certain types of nephritis the kidneys become very hard, indicating congestion and poor renal circulation. Winton (1933) theorized that if the mammalian kidney had no capsule and, therefore, had a lower intrarenal pressure (we shall abbreviate this term henceforth as IRP), the normal renal blood flow would be considerably greater. Weinstein and Adams (1943) recently suggested that the anuria accompanying sulfathiozole poisoning is due to an increase in IRP. Peters (1945a) expressed the opinion that increases in IRP might cause the oliguria of mercuric chloride poisoning, crush syndrome, transfusion kidney, and congestive heart failure. In another report Peters (1945b) lists thirty-two diseases which may lead to death from uremia and which he believes might all be caused by an increased IRP. In a recent review, Abeshouse (1945) states that surgical renal decapsulation has sometimes been used as a treatment for hypertension, especially in Europe.

In spite of the supposed importance of the influence of IRP on renal function, there have been only a few attempts to measure this pressure. Brodie (1914) made the following brief statement, "I made some attempts to measure this latter [IRP] during active diuresis, but at present have not obtained any very accurate results. As far as they go they indicate a tension of about 40 mm. Hg." Peters (1945a) reported that "animal experiments" indicated that decapsulation of the kidney results in a 50% decrease in IRP, but he neither described the nature of the experiments nor did he elaborate on his results.

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The only systematic attempt to measure the IRP is that of Winton (1931a, 1931b, 1933, 1934, 1936, and 1937). Using the isolated perfused kidney, he (Winton, 1931a) found that "the ureter pressure may often be increased 10 mm. Hg. or so without a noticeable diminution of urine flow." He (Winton, 1931b) also reported that an increase in the renal venous pressure of the isolated perfused kidney produced no effect on urine flow unless this pressure was increased by about 10 mm. Hg. He (Winton, 1933) explained these results by assuming that there was an IRP which must be exceeded before there is any effect on urine flow. He was making, thus, an indirect measurement of the IRP. He found that the normal IRP of the isolated perfused kidney averaged 10 mm. Hg., with a range of 4 to 14 mm. Hg. In another experiment, Winton (1937) found that qualitative changes in kidney tenseness, as measured by a sclerometer, varied with changes in IRP. Herman and Winton (1936) added further evidence to the quantitative estimation of IRP by the following experiment: they cannulated the renal vein, artery and ureter and then placed the kidney in an airtight chamber, running the cannulas through the stopper of the chamber. Meanwhile, the kidney was perfused as usual. Then, by lowering the chamber pressure below atmospheric, they could decrease the extrarenal pressure. With this method, they found that urine flow increased as the extrarenal pressure decreased, but that negative pressures below —10 mm. Hg. did not give further increases in urine flow.

Winton's work on IRP has done much to focus attention on the possible consequences of an increased IRP (Winton, 1937), but there is still much to be learned. All of Winton's work on this subject has been done on the isolated perfused kidney. This preparation has yielded much valuable information, but it is by no means a normal kidney. It is important that IRP be studied in the intact animal. Also, the IRP should be measured directly and not by such indirect methods as Winton employed.

In this laboratory we have (Bush, Coffman, Montgomery and Swann, 1949) attempted to avoid these objections by measuring the IRP of living dogs. The tissue tension method of Henderson, Oughterson, Greenberg and Searle (1936) was employed, this having originally been designed to measure the

"intramuscular pressure." The method has been described in detail in a previous report (Bush, Coffman, Montgomery and Swann, 1949). Briefly, it consists of a determination of the pressure required to force, in about 5 seconds, one or two cubic mm. of physiological saline through a syringe needle thrust into the kidney. The average pressure in 32 adult dogs under sodium pentobarbital anesthesia was found to be 72 mm. Hg. with a range of 55 to 88 mm. Hg. It was found to vary with hemorrhage, venous occlusion, diuresis, etc. Although this method was a direct approach to the problem, it gave results which were considered by the authors to be "ludicrously high." It was thought that the technique measured two characteristics of the kidney: a distortion pressure, which was compared with the pressure needed to spring in the bottom of an oil can, plus fluctuating intrarenal pressures. In order to avoid misunderstanding, Bush, *et al.* (1949) chose to call their measurement "FFP" (Force-Fluid-Pressure) rather than IRP.

Obviously, a more accurate method of measuring IRP is desirable. Now, intramuscular pressures have often been measured by setting a cannula in the tissue and then determining the pressure needed just to start fluid moving through the cannula into the tissue. Large volumes of fluid—2, or more, cubic mm.—are introduced in the method of Henderson, Oughterson, Greenberg and Searle (1936), which was used by Bush, *et al.* (1949) and also by Burch and Sodeman (1937), Gunther, Engleberg and Strauss (1942), and Kerr and Scott (1936). But McMaster's (1941b) experiments have cast some doubt on this technique, because he has shown that the quantity of fluid moving into the tissue, per unit time, is a function of the pressure, and that if one observes the movements of only very small volumes of fluid, very low "tissue pressures" are observed. We therefore selected a technique like that of McMaster (1941a) for these investigations, making provision to measure the flow of very small volumes of fluid out of the cannula into the kidney or vice versa, when various pressures were superimposed on the fluid in the cannula.

Methods

The apparatus used for measuring IRP consisted of a 27-gauge needle whose pointed end was closed with solder. This

prevented plugging when the needle was inserted into the kidney. Three small holes were filed in the side of the needle as close together as possible and about 3 mm. from the soldered end. This needle was thrust deep into the kidney parenchyma. Dogs under sodium pentobarbital anesthesia were used throughout these experiments.

The needle was sealed in a No. 5 ureteral catheter with Pyseal cement. The opposite end of the catheter was cemented to a glass capillary tube with a 0.5 mm. bore. The opposite end of the capillary tube was joined to one branch of a T-tube; the second branch of the T-tube was connected to a water manometer, and the third branch was connected to a 20 cc. syringe whose piston could be manipulated by an adjustable screw. Isotonic saline filled the apparatus from the needle openings to the middle portion of the capillary tube. Air filled the rest of the system from the meniscus in the capillary tube to the water manometer on one outlet of the T-tube and the syringe piston on the other outlet. The meniscus in the capillary tube was observed by a microscope whose eyepiece contained a calibrated micrometer. With this arrangement, the movement of the meniscus could be observed, and the flow of fluid, whether into or out of the kidney, was ascertained. The flow over a 5-minute interval was observed. Since the diameter of the bore of the capillary was known, the volume of fluid flow in the capillary was readily computed from the linear movement of the meniscus. A movement of 0.01 cmm. of fluid could readily be observed with the apparatus. The pressure in the system was then increased by from 3 to 8 mm. Hg. by means of the screw adjustment on the syringe piston, and, again, the movement of the meniscus was observed for 5 minutes. This procedure was repeated until the movement of the meniscus toward the kidney was so rapid that it could no longer be observed with any degree of accuracy.

Results

Determinations of the fluid measurement into, or out of, the kidney under various pressures were made in 9 dogs. Figure 1 shows the results obtained in four of these experiments. The other 5 dogs in this series gave, essentially, the same results.

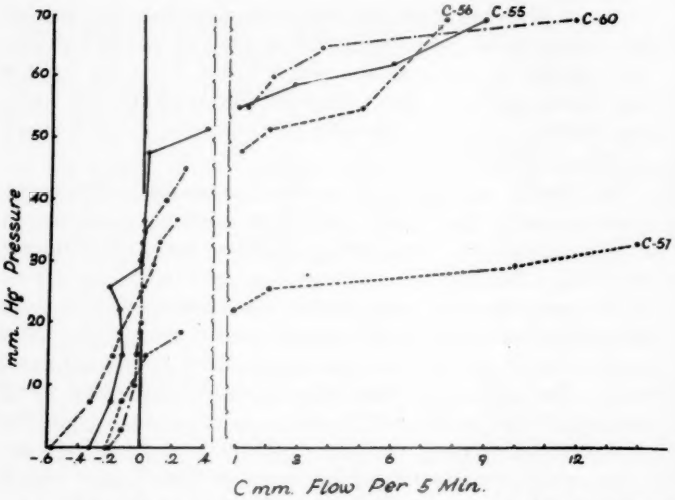

FIG. 1. Relation of pressures to fluid flows into or out of kidney. Negative values indicate flows out of kidney; positive values indicate flows into kidney.

The abscissa of the chart represents the rate of flow of saline into, or out of, the kidney in cubic mm. per 5 minutes. The negative values represent flow of fluid out of the kidney; positive values represent flow of fluid into the kidney. The ordinate represents the pressure throughout the apparatus.

In all dogs, when the fluid in the needle was under a pressur of 10 mm. Hg., or less, the meniscus of the capillary moved away from the kidney, *i.e.*, the fluid in the kidney was forced out through the needle. As the pressure was raised in the needle, the flow out of the kidney was first slowed, and then with further increases in pressure the flow was reversed in direction, and fluid was forced into the kidney. The point at which the flow was zero, by actual measurement or by interpolation, was 13, 25, 35, and 36 mm. Hg. in the 4 dogs. In the 5 dogs not shown in the graph the zero point was 26, 20, 20, 21, and 40 mm. Hg. The average was 26 mm. Hg. for the 9 dogs.

As the pressure in the needle in the kidney was further increased, the rate of flow out of the needle increased. Then,

abruptly, the pressure-flow curve showed a sharp "elbow," with further increments in pressure giving very large increases in flow. This breaking point appeared for the 4 dogs shown in the graph at roughly 60, 52, 49, and 23 mm. Hg. pressure and in the remaining 5 dogs at 50, 65, 53, 44 and 74. The average was 52 mm. Hg.

Discussion

The pressure which just prevents fluid from moving out of a small needle inserted in the kidney parenchyma has been measured and found to average 26 mm. Hg. This is a sharp contrast with the pressure found with another technique: we (Bush *et al.*, 1949) found it to average 72 mm. Hg. when the pressure which was required to force 1 or 2 cmm. of fluid into the kidney in 5 seconds was ascertained. The difference is due to the technique employed: as discussed by Bush *et al.* (1949), undoubtedly a considerable distortion of the tissue is caused by the incoming fluid and the technique thus measures two forces: a distortion pressure and other superimposed pressures. In fact, the distortion pressure is roughly defined by the present experiment: it is in the neighborhood of 50 mm. Hg., the difference between the measurements made by two techniques.

The present technique is thought to give a true measurement of the intrarenal pressure. But one can readily ask: where is this pressure being measured: is it cortical or medullary? is it interstitial? is it intratubular? An answer to these questions will be attempted in another paper. At any rate we shall henceforth, for the sake of brevity, designate the measurement as "IRP"—the intrarenal pressure.

The method originally employed in this laboratory has been discarded, because the distortion factor appears to be both unpredictable and relatively unimportant. It is apparent from the curves shown in Figure 1 that after the "breaking-point" is reached (at an average pressure of 52 mm. Hg.), the pressure flow relation is not constant from dog to dog. Sometimes very small increments in pressure give large flows, sometimes only small flows. It is therefore obvious that observations depending on movement of fluid into the kidney would not give very accurate results. The "FFP" end-point used by Bush *et al.*

(1949), was 1 or 2 cmm. in 5 seconds as observed by the naked eye. This comparatively rapid rate of flow naturally would give a very high pressure. This error would be permissible if the slope of each line from the breaking point in the curve were constant. This would mean that the error would be constant, and the "FFP" minus this constant would give a good index of the actual IRP. However, the slope of the lines varied widely among the animals studied by this method. From these results it is concluded that the values obtained by the method of Bush *et al.* (1949), were valid only in crudely representing rises and falls in IRP.

Since the results reported in this paper were obtained by actually balancing the pressure of the fluid in the kidney with a fluid under a known pressure, it seems that this method is a direct method of determining the IRP. The method is not without its objections, however. The rates of flow of fluids involved in the measurement are so small that from 15 to 45 minutes of observation are required to get a single reading. In this interval of time it is possible that the IRP might change spontaneously. Also, the previous work done in this laboratory indicates that very rapid changes in IRP occur under certain conditions; therefore, for the purpose of analyzing the dynamic changes in IRP the microscopic method is of little value. Experiments which are now in progress at this laboratory are attempting to overcome this difficulty.

The tissue pressure in relaxed muscles, using this same technique was found by Wells, Youmans and Miller (1938) to vary with different muscles, being about 2 mm. Hg. in muscles like the gastrocnemius with loose fascial covering and 5 mm. in the soleus which is invested with a tight fascial sheath. The same authors found the subcutaneous tissue pressure to be about 3 mm. Hg.; McMaster (1946) reported the intradermal tissue pressure to be about 2 mm. Hg. It is apparent that the tissue pressure in the kidney is markedly greater than in the other tissues so far investigated: it is about 10 times greater than that of relaxed muscle. It is about equal to the intramuscular pressure of the gastrocnemius muscle (Henderson, Oughterson, Greenberg and Searle, 1936) when

this muscle is maximally tensed; it is twice as great as the intramuscular pressure of the maximally tensed rectus femoris (Wells, Youmans and Miller, 1938).

SUMMARY

The intrarenal tissue pressure was determined by measuring the pressure required just to prevent fluid from flowing out of a small cannula set in the renal parenchyma. It was found to average 26 mm. Hg. in nine dogs, the range being 13 to 40 mm. This is about ten times greater than the tissue pressures observed in subcutaneous, intradermal or intramuscular tissues.

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THE ARMY MEDICAL LIBRARY'S PUBLICATION PROGRAM

FRANK B. ROGERS AND SCOTT ADAMS*

The Army Medical Library has announced its intention to develop a publication more nearly meeting 20th century needs than the *Index-Catalogue of the Library of the Surgeon General's Office*. This is a major decision affecting the bibliography of the medical sciences. For this publication and the Library which sponsored it were called by Dr. William H. Welch of Johns Hopkins University the most important of the four great contributions of the United States to medical knowledge. Its reputation is international; wherever it has been distributed it has brought credit to the Library and to American medicine.

Why should the *Index-Catalogue* be terminated? If it is a matter of funds, they can be found, as they have for the last seventy years; if a sufficient number of skilled personnel is not available, perhaps some means of co-operative preparation of the *Index-Catalogue* can be evolved.

Both of these considerations are pertinent but the primary reason for establishing a new publication program is that the *Index-Catalogue*, as a vehicle of publication, is no longer able to meet the demands of mid-20th century medical science.

John Shaw Billings (1838–1913) and the Index-Catalogue

To Dr. John Shaw Billings will always belong credit for having created the most monumental medical bibliography the world has known. He brought to the Surgeon General's Office in 1865 tremendous abilities, a high purpose, and a personal exasperation at the unorganized status of medical writings. When he left the Library thirty years later, he left as two of his accomplishments the world's greatest collection of medical literature and a bibliographical key of comparable magnitude, the *Index-Catalogue*.

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*Major Rogers is Director of the Army Medical Library, and Mr. Adams is Assistant to the Director. Received for publication January 30, 1950.

It is pertinent to inquire into two matters concerning the origin of the *Index-Catalogue:* what was the nature of the medical community for which Billings planned his work; and what purposes did he have in mind in planning it?

The year 1876, year of the International Congress of Medicine held at the Centennial Exposition in Philadelphia, saw American medicine between two worlds. The new advances of Virchow in cellular pathology, of Pettenkopf in preventive medicine, of Nightingale in hospital care, caused an intellectual ferment which reached to American shores. Medical schools were lengthening their courses and were building laboratories for students and professors. Hospital facilities for teaching were becoming an established practice. Students were visiting Europe for post-graduate study, and for the first time a woman attended the sessions of the American Medical Association.

However, the old held on. American medicine was still predominantly individualistic medicine, practiced by lone physicians in rural and semi-rural communities. The mold of the frontier, like that of colonial days, enforced individualistic practice. Research work was necessarily limited to a few cities on the eastern seaboard; even there Virchow and Koch were not always heroes to all physicians. The International Congress of 1876 heard papers showing that results equal to those of Lister could be had without the "folderol" of Lister sprays and antiseptic gauze packs. Older members of the profession deplored the trend to specialism and practiced medicine similar to the calomel-quinine-diet medicine of Rush and Bard.

But seven State Boards of Health had been established. Public Health Departments, where they existed, were little more than inspection groups closely allied to quarantine stations. When Dr. Gilman Chandler wished to work on home sanitation and disinfection for the New York City Board of Health he had to do it in his capacity as Professor of Chemistry of Columbia College. Almost no publicly supported research laboratories existed, and certainly no funds for research in private institutions came from the public treasury. Government was of little consequence in medicine; research and resulting publications represented largely the product of individuals working and writing alone.

Throughout his career Billings insisted on the practical advantages which the *Index-Catalogue* might have for the medical profession. In the *Specimen Fasciculus*[1] of 1876, he hoped that it would be found "of practical interest and value in a bibliographical point of view as well as for the immediate use of the collection." Again if the entire catalog could be published no collection of the Government would "be more used or be of more practical utility . . . (it would) soon tend to elevate the standard of medical education, literature and scholarship of the nation, and . . . thus indirectly be for the benefit of the whole country, since the general knowledge and skill of the medical profession become a matter of personal interest to almost every individual at some time during the course of his life."

His often-quoted speech before the Medical College of Ohio in 1888 reiterates these practical considerations. Difficulties in the search he had made for literature relating to his dissertation on surgical treatment of epilepsy convinced him that libraries and bibliography must be organized to advance the medical sciences. Billings was not a bibliographical dilettante; he approached bibliography with a professional pragmatism and a deep-seated conviction that the library and its *Index-Catalogue* must be useful in the cause of medicine.

Billings' purposes were practical. He set out to provide a medical world, finite and relatively stable by our standards, with the bibliographical equipment it needed to do its work. It was a world whose demands were modest. Contributors to medical science worked alone—without financial assistance from foundations or government, without elaborate organizations created to insure the publication of the results of applied and basic research.

Antecedents of the Index-Catalogue

The first major bibliographical effort of Dr. Billings was prepared and printed in six months to meet a want which daily became more pressing. It was the one-volume catalog of 1872,[2] containing 13,000 entries for monographs, and a subject index of 21 pages referring to the author entries. This subject index is the prototype of medical bibliography in the Army Medical Library.

This Catalog, however, was merely the preliminary edition of the two-volume author catalog of 1873–74.[3] With its supplement of 1874 containing entries for anonymous works, associations, societies and periodicals, it represented the *author* key to the collection. Generally unrecognized is the fact that he was busily engaged in supplementing these volumes with a separately prepared and published *subject* catalog. The dictionary form of the *Specimen Fasciculus* and the 1880 *Index-Catalogue* represented a later amalgamation of this separate subject catalog with his author entries.

Billings first describes this work in 1874.[4] In 1875[5] he amplified his earlier announcement: "The Subject Catalogue, including references to the principal original papers in medical journals and transactions, is far advanced in preparation; and even in its present incomplete form has been found of much value, not only for the work of this Office, but for the benefit of medical writers of this country, who must refer to this collection for much information and literature which cannot be obtained elsewhere in the United States, and whose inquiries could not be answered without some such systematic index."

By 1878, indexing back files and current journals, he had accumulated 400,000 subject entries for publication.[6] Parenthetically, it may be observed that this prepublication accumulation set the pattern for backlogs of unpublished material which have never been eliminated.

The Index-Catalogue

It is interesting but profitless to speculate on the course of the Library's bibliographical history if the Subject Catalogue had been published separately. We know that Billings consulted widely with both physicians and librarians, and that he had virtually decided on the dictionary form by 1876 when the *Specimen Fasciculus* was published. The *Index-Catalogue* was to be "a combined alphabet of subjects and authors, arranged in dictionary order under a single alphabet."

Actually, the order of subjects represents a compromise between alphabetical and classified arrangement; the *Index-Catalogue* is an alphabeto-classed catalog. Subordinate headings are

grouped schematically, often by inversion, under the major subject headings.

Billings, who exhorted his colleagues for so many years to give their papers accurate titles, was scrupulous in naming his own publication. The *Index-Catalogue* was the catalog of the library, and the index to the polygraphic journals contained in the library. "Permit time," he said in 1880,[7] "to call attention to the fact that this is not a complete medical bibliography, and anyone who relies on it as such will commit a serious error. It is a Catalogue of what is to be found in a single collection,—a collection so large and of such a character, that there are few subjects in medicine with regard to which something may not be found in it, but which is by no means complete."

Contrary to popular conceptions, it is not a complete index to the scientific literature in the Library. Billings indexed for the "*principal* original papers in medical journals and transactions,"[8] he gave short shrift to papers he considered to be of secondary importance, and he scorned to include many journals as beneath his notice. Garrison,[9] writing in 1929, stresses this point: "It has been a matter of common error to suppose that the *Index-Catalogue* contains, in addition to medical books and pamphlets, all the articles contained in the medical periodicals in the Library. . . . This is, and has been, wide of actual fact. In the period of inception (1865–1879) Billings personally checked all the periodicals in the Library for indexing, a colossal undertaking, done at his home into the small hours of the night. After the arrival of Fletcher, he continued to do this up to his retirement from active duty in 1895. . . . The two men undoubtedly checked all the articles in the purely scientific periodicals, such as *Virchow's* or *Pflüger's Archiv*, and all the important articles in weekly and provincial medical periodicals, such as the *Lancet* or the *Lyon Médical*. But there are some curious omissions here and there. A random examination of Vol. 1 of the *Lancet* for 1868, for instance, shows the omission of fairly important clinical cases (printed nowhere else) on pp. 314, 315, 376, 558, 588, 589. . . . For medical journals of third, fourth, or fifth rate type, Billings showed little consideration, passing them by if their contents were worthless or otherwise acting as

if on Osler's view of the medical journals of Australasia in 1897 —that they contain little except records of hydatids and snakebite. Following Billings' retirement in 1895, the medical periodicals have been checked for indexing by the successive Librarians and along the established lines indicated above (omission of trite, insignificant or worthless material).

"In selecting material for the *Index Medicus*, Fletcher tended to include more, on the ground that current articles of any kind are apt to be of current interest to current readers. . . . With the better sort of medical journals, his slogan was 'take everything.' The *Index Medicus* is therefore more complete as a record of this kind than the *Index-Catalogue*, which Billings aimed to make a repository of the very best and most select material, but of no other."

The *Index-Catalogue* started, therefore, as a book catalog of the monographs and a selective index to the periodicals contained in a single collection of 52,000 volumes. It was cast in dictionary form for the convenience of the user who wished to learn what was included in that collection. It shared the weakness of all book catalogs of growing collections; additions by interpolation are impossible; new series are inevitable.

In the case of the *Index-Catalogue*, this weakness is magnified out of all proportion by the fact that the publication attempted to catalog individual papers appearing in serials as well as monographs. Thus, by 1948 the *Index-Catalogue* had listed 2,457,693 papers under subject, as compared to 407,508 monographs.

Throughout the late nineteenth century the journal gained in popularity as a vehicle for scientific publication, but not until the turn of the century did it virtually crowd out the monograph. The following table tends to reflect the difference between the character of medical literature of Billings' day and the twentieth century:

INDEX-CATALOGUE

Subject Entries

	Monographs	Serials	Total	Percent Serial
First Series	168,537	511,112	679,649	75%
Second Series	136,405	645,557	781,962	83%
Third Series	57,034	603,600	660,634	91%
Fourth Series (through V. 10)	58,132	697,424	755,556	92%

Original Estimates

Billings' original estimate of the size of the *Index-Catalogue* and the subsequent revisions necessitated by continuing indexing are pertinent. In 1876[10] he estimated that "the complete catalog on the present plan (of the *Specimen Fasciculus*) would make five volumes of about one thousand pages each." In 1878,[11] still previous to publications, he revised this estimate. The 400,000 subject references on hand, he said, "would make seven volumes in royal octavo of about 1,000 pages each. The author catalogue would make an additional three volumes if published separately."

The original estimate of five volumes was exceeded threefold in fact. The first series comprised 679,669 subject references (not 400,000) in sixteen volumes. At the close of the first alphabetical series, although the Library had grown to 308,000 volumes, Billings still had not abandoned the concept of a finite catalog; he announced that the manuscript of a second series had been prepared, and would probably make five printed volumes of a format similar to that of the first.[12] His estimates were again wide of the mark. The estimated five volumes for the supplement to the First Series, under the editorship of Dr. Robert Fletcher, became the 21 volumes of the Second Series.

By virtue of the fact that it was a catalog the *Index-Catalogue* was committed from its beginning to publication in alphabetical order. So far, its history parallels that of traditional book catalogs for whose subsequent volumes and series the world patiently waits. By virtue of the fact that it cataloged papers as well as monographs, it built up huge reservoirs of unpublished references which forced it to become a serially published catalog.

The fact that it is a catalog of scientific papers which require rapid communication has produced a series of crises in its publication history. Book catalogs of libraries devoted to the humanities and social sciences can, with some ill grace, submit to leisurely publication. A book catalog in which 90 percent of the contents is scientific serial literature is faced with an urgency of publication of an altogether different character.

The Index Medicus

Billings did not intend the *Index-Catalogue* to meet the demands placed on the Library for a serial index to currently published literature. This was to be the function of the *Index Medicus*,[13] the necessary complement to his repository catalog. It was the purpose of the *Index Medicus* "to record the titles of all new publications in medicine, surgery, and the collateral branches, received during the previous month. These will be classified under subject headings, and will be followed by the titles of valuable original articles on the same subject. . . . The periodicals thus indexed will comprise all current medical journals and transactions of value, so far as they can be obtained."[14]

The *Index Medicus* began its career under a commercial publisher, F. Leypoldt, in 1879. After six years of publication, Billings and Fletcher announced its discontinuance in 1884. Another commercial publisher, G. S. Davis, was found who struggled with the fiscal problems of publication until 1895 when Rockwell and Churchill took it over, only to suspend in 1900. From that time until 1903, when Billings was able to secure a grant from the Carnegie Institution of Washington, there was a gap filled only by the three-volume *Bibliographia Medica (Index Medicus)*, Paris. With Carnegie funds the *Index Medicus* continued its publication based on the Army Medical Library until 1927, when it merged with the *Quarterly Cumulative Index*, started by the American Medical Association in 1916, to form the *Quarterly Cumulative Index Medicus.*

From 1927 to 1931 the *Quarterly Cumulative Index Medicus* was a co-operative production of the Army Medical Library and the American Medical Association. After January 1, 1926, currently published journal articles were no longer listed in the *Index-Catalogue*, but were published in the *Quarterly Cumulative Index Medicus.* Working difficulties interfered with close co-operation and from 1932 until 1941 the *QCIM* published by the American Medical Association was the sole index to current medical literature published in this country.

The career of the *Index Medicus* was beset with fiscal problems. It was conducted without governmental sponsorship, as

a free-time labor of Dr. Robert Fletcher. Its publication difficulties were tremendous; that it survived at all is due to Fletcher's courage.

Why Billings sought public funds for the *Index-Catalogue* and not for the *Index Medicus* is not known. Again it is interesting to speculate on the course of medical bibliography if government funds had been used to subsidize the life of the *Index Medicus*. At any rate, its transfer to the American Medical Association in 1927 left the Army Medical Library with but one bibliographical contribution: the *Index-Catalogue*.

Fourteen years later a second was added. The *Current List of Medical Literature*[15] began publication at the Army Medical Library in 1941. It was sponsored privately by the Friends of the Army Medical Library, organized by Dr. Atherton Seidell. Dr. Seidell convinced the Library that the medical sciences needed a more rapid means of communication than was afforded by the *Index-Catalogue* or the *QCIM*, and that the advent of photocopying services in libraries called for a weekly index to provide for prompt dissemination of information concerning articles available on microfilm.

The *Current List* was officially made a government publication published by the Library in 1945. It depended wholly on duplicates of the index cards prepared with the *Index-Catalogue* and it relied on offset printing of typewritten copy for speed in publication. By 1950 the *Current List* covered approximately 70,000 current articles (compared to the 225,000 of all dates selected for indexing by the *Index-Catalogue*).

Proposed Suspension of the Index-Catalogue

The continuation of the *Index-Catalogue* has long been in question. Following Dr. Fletcher's death in 1912, Dr. Fielding H. Garrison assumed the editorship of the *Index-Catalogue*. Both Fletcher and Garrison carried out the basic plan of publication established by Billings in the *Specimen Fasciculus*. It was not until 1921 that this plan was threatened.

Following publication of the third volume of the Third Series in 1921, General Robert E. Noble, the Librarian, submitted to the Surgeon General, with his annual report,[16] a separate report "with certain recommendations for changing the method

of publishing this volume." This original report cannot be found, but in 1922 he amplified his statement: "With the increase in the output of medical literature in all classes, there is a corresponding increase in the number of items indexed. During the present fiscal year more than 75,000 items were indexed, classified, and filed. In order that the more than 1,000,000 cards on file in the Library of the Surgeon General's Office may be published and the catalogue finally converted into an annual rather than a serial, it is very necessary that sufficient funds be obtained to publish an annual volume of the *Index-Catalogue* that contains not les than 150,000 items until all are published. The cost will be approximately $22,000 a year for nine years. Thereafter the cost would be materially less, as the annual would contain about 75,000 items. The third volume of the Third Series is the only volume within recent years that has published more items than were indexed during the fiscal year of publication. The funds alloted for the publication of the *Index-Catalogue* for the fiscal year 1922–23 are sufficient to publish only about two-thirds of the items indexed and classified during the fiscal year 1921–22."[17]

General Noble repeated his plea in his 1923 Annual Report, and in the letter of transmittal accompanying the third volume, suggesting that the volumes completing the Third Series might contain, if funds permitted, up to 200,000 entries, he added "each subsequent volume (after the Third Series) would be an annual catalogue of current medical literature instead of a catalogue of material which has accumulated in the preceding 25 years." He believed that conversion of the catalogue into an annual would add immensely to its value (Report of the Surgeon General, 1923).[18]

No action followed General Noble's proposal until 1926, when Colonel James M. Phalen, then Librarian, announced in his letter of transmittal accompanying Volume VI of the Third Series: "Beginning with Volume VI, the material catalogued under subject titles covers only the period prior to January 1, 1926. Author titles covering books and pamphlets are indexed up to date. This arrangement will be continued until the completion of the Third Series. Subject titles omitted from this publication can be found in the *Index Medicus* for 1926, and

in the *Quarterly Cumulative Index Medicus* for subsequent years." The proposed change was initiated by the Surgeon General in the interests of economy.

This newly announced policy met with opposition. Colonel Percy M. Ashburn, writing his transmittal letter for the last volume of the Third Series in 1932, had this to say: "As stated in the earlier volumes, it was at one time planned to close the *Index-Catalogue* with the Third Series, which was to include nothing appearing after 1926. In response to a very general demand by libraries and research institutions, that plan has been changed and work on the Fourth Series will begin at once. . . . All material appearing between (1926) and the present will appear in the next Series."

The reversal of this decision had the practical effect of adding to the extreme difficulties of publication. The subject entries accumulated between 1926 and 1932 were added to the estimated 1,000,000 unpublished cards on hand. During the depression years no funds were available to print copy from 1932 until 1936 when the Fourth Series was launched.

In 1938 Colonel Harold W. Jones, attempting to find a solution to this problem of accumulated unpublished references, in his letter of transmittal accompanying Vol. III of the Fourth Series, proposed the regular annual publication of alphabetical volumes of 1,100 pages each to include 90,000 references. This involved the preparation for printing of 2,000 references per week. Volumes of the Fourth Series were published annually until 1943 (although but two reached the 90,000 mark). Volume IX was published in 1945; Volume X in 1948; and Volume XI will presumably be issued in 1951.

On 24 May, 1944, Colonel Jones called a conference of consultants, including representatives of the professional library group then surveying the Library, to consider the problem of publication. This conference considered the possibility of adopting a 12-year cycle for each alphabetical series and of printing 120,000 references or 1,500 pages a year. The meeting was preceded by a questionnaire sent to the medical and library professions. It was agreed that indexing done by the Library should be more selective. Dr. Morris Fishbein, representing the American Medical Association, mentioned his hopes that

there might be an annual index to the world's medical literature, combining the activities of the *Index-Catalogue* and the *Quarterly Cumulative Index Medicus*.

Concurrently the Library was being surveyed at the request of the Surgeon General by a group of professional librarians headed by Keyes D. Metcalf, Librarian of Harvard College Library. The *Index-Catalogue* and its operations were closely scrutinized by the Survey Committee; selection and arrangement of subject headings, use of foreign nomenclature and the organization of the work were the foci of critical attention. The printed Survey Report[19] contains a number of suggestions for the future of the *Index-Catalogue*.

The Committee suggested *inter alia* the establishment of an Editorial Advisory Committee to insure continuity of policy and periodic review. It suggested that indexing be co-ordinated with the American Medical Association, ultimately resulting in a single service "issued in not less than monthly parts, and cumulated up to at least five year volumes."

The Committee also made recommendations on the coverage of literature, on the choice of subject headings (which were to be brought gradually into conformity with the American Medical Association's to insure ease of later co-ordination), and on publication procedure.

The Committee of Consultants on Indexing

It was not until 1947 that steps were taken to implement the Survey Committee's recommendations. Colonel Joseph H. McNinch suggested to the Executive Committee of the Association of Honorary Consultants to the Army Medical Library on May 27, 1947, that an advisory committee of consultants on the indexes published by the Army Medical Library be appointed. The response was enthusiastic and immediate. Both Dr. Morris Fishbein[20] and Dr. John F. Fulton[21] registered their approval of a comprehensive index published monthly with annual, and possibly quinquennial, cumulations.

The Committee of Consultants for the Study of the Indexes to Medical Literature Published by the Army Medical Library was established on July 7, 1948. Its original membership was carefully selected to represent the best thought on medical

bibliography available. The personnel of the Committee is as follows:

Dr. Lewis H. Weed, Chairman (to Sept. 29, 1949)	National Research Council
Dr. Basil G. Bibby (appointed Sept. 29, 1949)	Eastman Dental Dispensary
Mr. Verner W. Clapp (appointed Sept. 29, 1949)	The Library of Congress
Miss Janet Doe	New York Academy of Medicine
Dr. Morris Fishbein	American Medical Association
Dr. John F. Fulton	Yale University
Dr. Ebbe C. Hoff	Medical College of Virginia
Dr. Sanford V. Larkey	Welch Medical Library
Dr. Chauncey D. Leake (Chairman from Sept. 29, 1949)	The University of Texas
Colonel J. H. McNinch, M. C.	Office of the Surgeon General
Dr. William S. Middleton	University of Wisconsin
Dr. Eugene W. Scott	U. S. Interdepartmental Committee on Scientific Research and Development
Mr. Ralph R. Shaw	Department of Agriculture Library
Dr. Mortimer Taube	U. S. Atomic Energy Commission
Major Frank B. Rogers, M. C. (ex officio)	Army Medical Library

In order to provide this advisory committee with a working group which might explore the problems involved in medical indexing a research contract was granted to Johns Hopkins University in October, 1948, by the Medical Research and Development Board, Office of the Surgeon General. Dr. Sanford V. Larkey heads this research group.

The Committee has held eight meetings between September 24, 1948, and March 17, 1950. It has explored systematically the questions of use of and need for medical indexes, and of techniques, including new machine methods, which might be used in reducing the labor of building them. It listened carefully to discussions by the editors of existing indexes, including the *Index-Catalogue*, of scope, coverage, policies, and operations. The Research Project at Johns Hopkins gathered opinion by interviews, studied coverage by the existing services, the theory and practice of subject heading and classification as applied to medical literature, and the application of machine methods.

Decision to Discontinue

At the sixth meeting of the Committee on October 20, 1949, Colonel Joseph H. McNinch, as outgoing Director of the Army Medical Library, and Major Frank B. Rogers, the incoming Director, suggested that the Committee devote its attention to the critical state of medical indexing in the Library. Both Colonel McNinch and Major Rogers included recommendations for the closing of the *Index-Catalogue* and the substitution of a new currently published index to medical literature built on the framework of the *Current List*.

A sub-committee was appointed to study these recommendations. The sub-committee made its report at the seventh meeting of the Committee on December 16, 1949. The following were its recommendations:*

1. The *Index-Catalogue* should be stopped as soon as it is administratively possible to do so. This means that the sub-committee feels that the publication of the present series should not be completed and that the volume now in preparation should be the last volume of the *Index-Catalogue*.
2. The Army Medical Library should continue to develop an indexing program of its current receipts of current material. Initially the publication from this indexing program could probably best follow the general pattern of the *Bibliography of Agriculture*, and might be developed from the *Current List*.
3. Consideration should be given to publication of a catalogue of selected monographic material from the backlog, including theses and dissertations in a dictionary arrangement by author and subject.
4. The present backlog of cards intended for use in future volumes of the *Index-Catalogue*, minus the cards for the monographic material already provided for, possesses values that should not be lost. Methods of utilizing these cards should be developed by the Army Medical Library.
5. Selected non-current monographic material to be received in the future should be included either in the current

*As finally approved by the Committee.

index or in some other catalogue of monographic material. Non-current serial publications should be carefully recorded as to whether or not they have been analytically indexed.

The recommendations were discussed by the full Committee and were approved. After the matter had been referred to the Surgeon General of the Army and his approval obtained, the Director of the Library announced that the *Index-Catalogue* would be brought to a close, and that a new type of publication would be substituted for the traditional publication in alphabetical series.[22]

Mathematics of Index Publication

This action by the Library followed thirty years of temporizing with an insurmountable problem. The Library faces today conditions analogous to those faced by General Noble in the first post-war period of 1920. Three factors are responsible for the crisis following World War II:

1. Publications from areas affected by the war, delayed for six years, have been received in huge quantities.
2. Post-war publication of scientific literature has increased tremendously.
3. The Library has pursued an aggressive program of acquisitions following the Survey, filling countless gaps in its files of older publications, and establishing thousands of serial titles for the first time.

These three factors have precipitated a situation in which, despite an all-time high in the indexing rate, there is a backlog of an estimated 65,000 unindexed *pieces* of serial literature. There is a further growing backlog brought about by the discrepancy between the rates of indexing (215,669 in 1949) and "classifying," or completing the asignment of subjects to cards (159,641 in 1949).

All this is but incidental to the problem of unpublished material. Discrepancies in the rates of indexing and publication have long plagued the *Index-Catalogue*.

In tabular form, publication of references and the residual files following publication are noted below:

	Published	Subject references unpublished at end of period
1878.....	.....	400,000
1880–1895.....	679,669	212,000 (est.)
1895–1920.....	848,285	1,000,000
1920–1950.....	1,337,247	1,750,000

Omitted from this last figure of 1,750,000 unpublished subject references are unpublished author entries for an estimated 150,000 monographs. Clearly the form of publication of the *Index-Catalogue*, designed by Billings to satisfy the modest needs of the medical community of 1876, has proved to be utterly incapable of sustaining the burden of mid-twentieth century publication. The graph "Projection of Indexing and Publication Rates" pictures this situation, assuming current rates, over the next ten years.

In this chart a constant indexing rate of 200,000 items a year is assumed. 215,000 items were indexed in 1949, and current indexing is at the rate of 264,000 items per year. 200,000 is therefore a reasonable and practical rate. Based on the present unpublished subject file of 1,750,000, this would result in a theoretical total of 3,750,000 references accumulated by 1960.

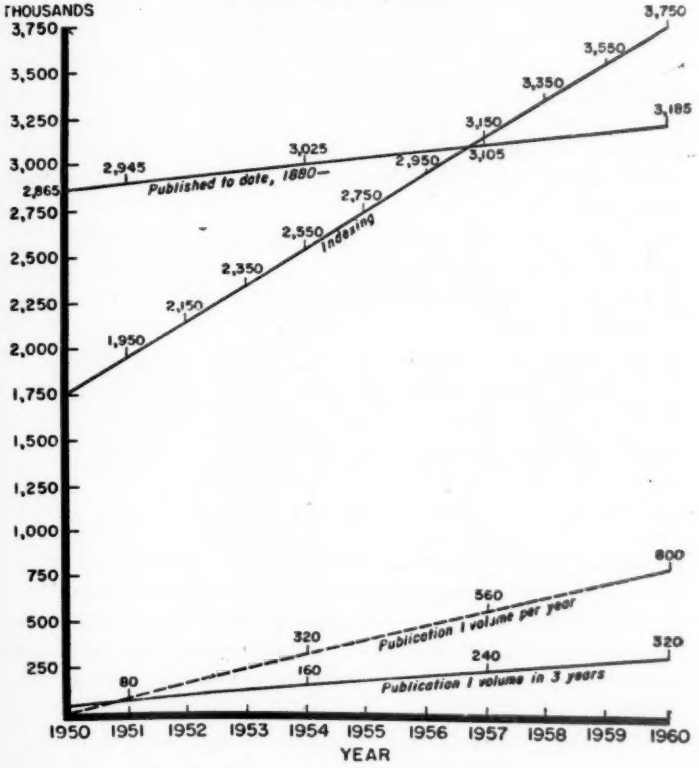
Publication of the tenth and eleventh volumes of the Fourth Series progresses at the rate of one volume of 80,000 references every three years. By 1960 the Library will be able to publish 320,000 subject references. The difference between the total cumulation and the total publication represents the real growth of the unpublished subject file.

Prior to the war, the *Index-Catalogue* achieved an annual publication rate of approximately 80,000 subject references. This rate (represented by the dotted line) is also shown. Since the annual differential at this rate results in a publication deficit of 120,000 cards, annual publication can no longer be considered the solution to the publication problem.

Of interest also are the publication totals of the *Index-Catalogue* from 1880 to date. By 1958, at the rate of one volume in three years, the *Index-Catalogue* will have published 3,105,000 references. The net contents of the unpublished subject file in that same year will be 3,110,000 references. In other words.

from 1958 on the *Index-Catalogue* will be withholding from publication more information than it has been able to publish in the 78 years from 1880 to 1958.

PROJECTION OF INDEXING AND PUBLICATION RATES
THE INDEX-CATALOGUE



Real growth of unpublished subject file:

	At one volume in 3 yrs.	At one volume per year
1950	1,750,000	1,750,000
1951	1,870,000	1,870,000
1954	2,390,000	2,230,000
1957	2,910,000	2,590,000
1960	3,430,000	2,950,000

Withholding of Information Indefensible

The deferring of 1,750,000 references from publication is neither scientifically nor morally justifiable. It is even less defensible when it is considered that the material deferred will be the recent articles, of immediate value to scientific research. Thus today the Library has an accumulation of 9,000 subject cards under Penicillin, including original papers from Fleming to date. It would not be possible to publish this bibliography until 1954, when the letter "P" would presumably be reached. In the meantime, it may be presumed that laboratory work would be duplicated, and thousands of dollars wasted in research which might have been saved if this information had been published.

Research cannot be tied to the alphabet, and yet this was the basic assumption underlying the *Index-Catalogue*. The alphabetical pattern established by Billings for a printed book catalog is today impeding scientific progress.

Were John Shaw Billings to be faced with this problem, is it not probable that he would have realized the implications of this situation thirty years ago, and devised a publication which would serve the twentieth century as well as the *Index-Catalogue* served the nineteenth? He had a strong utiltarian sense and would have wished to make his index scientifically useful. However, his successors have faithfully followed the original alphabetical pattern. When they wished to break away, they have been faced with problems of cost, problems of co-ordination with other services, problems raised by staggering backlogs of unpublished references, and by emotional arguments raised by more tradition-minded members of the medical profession.

In all this consideration of quantities of references to be published, the estimates have been based on the traditional *Index-Catalogue* practice of *classifying* references for publication. Each of the subject references to books and articles has appeared once only under a specific subject. This practice raises two further considerations. First is the desirability of multiple entry, or the listing of a single article under several subject headings. Scientific literature is written more frequently about

the relation of A to B, or the effect of A on B, than it is about either A or B as separate entities. It is highly desirable in its indexing, therefore, to reflect the multiple facets of scientific thought, to make the articles available to a wider group of inquirers through providing multiple approaches. The *QCIM*, for example, averages two and one-half subject headings per article. This cannot be done with the *Index-Catalogue* in its present form. Re-examining 1,750,000 books and articles headed over a 30-year period in order to add additional headings is an impossible task.

The second consideration is that the practice of classification results in an effective device for concealing rather than disclosing medical literature. There are at present 90,000 unpublished references to the literature of Tuberculosis. By the time the letter "T" is reached in 20 years, this total may well be 140,000. This is sufficient to make two 1,000-page volumes in the present close-set style of the *Index-Catalogue*. These 2,000 pages will necessarily have to present the most elaborate schematic breakdown of the subject that the world has seen, and in this labyrinth of subject groupings each of the 140,000 references will appear once, and once only. This is truly "Fort Knox bibliography"—the laborious mining of references, to bury them securely again.

Use Studies

During the last two years two use studies of medical indexes, including the *Index-Catalogue*, have been made. The first in point of completion was made by Dr. Chauncey D. Leake,[23] who circulated a questionnaire among the medical librarians at the meeting of the Medical Library Association in Galveston, April 11–14, 1949.

Dr. Leake's questions called for statements of preference for an index to medical literature. A total of 73 librarians indicated their choices in the following order.

Choice	1	2	3	4	5
Index-Catalogue	3	23	23	6	6
Comprehensive Cumulative	53	11	3	3	0
Selected Cumulative	3	4	13	11	23
Specialty Series	0	2	5	27	18
Current List	15	25	15	4	4

Thirty-five of the 73 replies indicated preference for quarterly appearance; 28 for monthly; five for semi-annually; and five for "frequently." This study, while not conclusive, indicates preference of a large group of medical librarians for a comprehensive medical index appearing quarterly.

A second use study was made by the Medical Index Research Project under Dr. Sanford V. Larkey.[24] Dr. Larkey, interviewing both medical workers and librarians, investigated the use of a number of individual indexing and abstracting services in the field of medicine. A comparison between the use of the *Quarterly Cumulative Index Medicus*, the *Index-Catalogue*, and the *Current List of Medical Literature* follows:

Medical Workers

	Use very often	Fairly frequently	Infrequently	Do not use
Q C I M	17	30	20	10
Index-Catalogue	6	8	25	25
Curent List	4	9	18	30
	Librarians			
Q C I M	28	8	1	0
Index-Catalogue	20	13	2	2
Curent List	8	6	14	9

While these figures are not conclusive they confirm common experience that the *QCIM* is preferred, and that the *Index-Catalogue* is more heavily used by librarians than by physicians.

Comments on desirable frequency of publication in Dr. Larkey's study indicate a strong preference, shared by both medical workers and librarians, for a monthly or a quarterly index.

Neither use study produced conclusive results. Each, however, indicates that there is a desire among users for a serial index appearing frequently in preference to the *Index-Catalogue* form of publication.

Cost

Thus far, evidence has been produced to show that the present form of publication for the *Index-Catalogue* is neither practicable nor desirable. Nothing has been said about the cost of this

enterprise. If the values are questionable, surely the price is significant.

The last volume of the *Index-Catalogue* (Vol. X, Fourth Series) cost approximately $175,000 for labor and printing alone. It was published in an edition of 1,000 copies for the Army. This means that each of the 1,000 copies of this 994-page volume cost the Department of the Army approximately $175.

The New Publication Program

As a result of studies made of medical and scientific indexing during the past few years, it is possible to formulate a statement of the general requirements for a modern medical index.

In the first place, a modern index *must* record the current literature. It is essential that day-to-day advances on the frontiers of scientific investigation be noted and brought to the attention of the scientific world. If an index can do more than this without detriment to its primary purpose so much the better, but its primary purpose remains the reporting of the results of contemporary research. New information is the life-blood of science.

No medical index now in existence has been able to adapt itself to the advent of new forms of primary publication and the tremendous increase in literature published in conventional forms. The results of work done today in laboratories throughout the world seep slowly through devious channels into the consciousness of the American medical investigator. Ignorance of successful experimental activity leads to duplicated effort and waste of funds, and delays the advance of medicine.

It is probably true that Medicine makes greater use of its past writings than do the non-biological sciences. Fortunately, however, Medicine is already provided with a bibliographical control over its earlier literature greatly in excess of that enjoyed by any other science. The 2,865,201 subject references in 57 volumes of the *Index-Catalogue* constitute a permanent body of reference covering publications of the 15th to the 20th century.

This generation must solve the problems of indexing contemporary publication, and this generation cannot evade the responsibility of providing for the future. As the present fades into the past an accessible and comprehensive record of its achievements

must remain. So far as bibliography is concerned, "if we take care of the present, the future will not have to take care of the past."[25]

The index to be published by the Army Medical Library will, therefore, be an index to the current literature of Medicine. As such it must satisfy four requirements which have emerged from the study, discussion and comment devoted to this problem. A medical index must be prompt and comprehensive; it must be convenient to use, and it must have expansibility.

Practicing physicians, research workers and librarians all agree that an index, in order to be useful, must appear promptly. Scientific information offers its greatest values when communicated without delay. The consensus of opinion seems to be that a medical index should be published at a monthly or quarterly frequency, and that the time lag between the appearance of an article and its appearance in an index should be kept at a minimum.

"Comprehensive" is a word of many meanings. Medical indexing never has been total; it cannot be total in the future. Questions of quality must inevitably be considered and selections must be made. Medical indexing should report medical and scientific literature of significance in research for the use of workers in Medicine and its specialties. Its scope should include important contiguous subject fields as well as core disciplines in Medicine. Its coverage of the significant literature within these fields should be thorough regardless of language.

A third desideratum is that of convenience to the user. Information should be presented in such a manner as to insure successful and rapid consultation. This implies that there should be multiple subject and author approaches. Furthermore, it is desirable that entries be grouped in lots convenient for retrospective search. The index should therefore be provided with a cumulative feature.

Adoption of a rigid format is to be avoided. The format should be flexible so that additional literature may be included as circumstances require with a minimum of administrative and technical difficulties.

These requirements have been carefully considered by the Army Medical Library in reconstructing the *Current List*.

Desirable features have been borrowed from other publications which have met some of these requirements successfully. In particular, the *Bibliography of Agriculture* published by the Library of the U. S. Department of Agriculture has influenced the Library's planning.

The new index will be published in monthly issues. Each issue will have two parts: a register section, in which the contributions are listed; an index section, where author and multiple subject headings will be interfiled in dictionary form. The monthly indexes will cumulate into annual volumes comprising entries for approximately 80,000 articles.

Promptness. The first issue for July 1950 will report literature as recent as April. Every effort will be made, by improving assemblage techniques, to reduce this time interval.

Comprehensiveness. The index will cover initially 1,225 selected journal titles. These titles will be indexed thoroughly and regularly. The titles have been selected on the basis of the proportion of original work reported in the journals, and on the pragmatic basis of journals most commonly held by American medical libraries.

Additions. The Library intends to increase the number of journals covered as rapidly as funds, personnel, and facilities permit. Newly appearing titles will be screened for selection, and the Library's basic holdings will be periodically reviewed for additions. An up-to-date list of the journals covered by the index will be published annually.

Languages. Of the initial list of 1,225 journals, 526 or 43 precent are in the English language, and 699 or 57 percent are in foreign languages.

The latter are distributed as follows:

	% of total		% of total		% of total
French	13.	Portuguese	3.6	Dutch	.5
Italian	12.	Mixed language	2.4	Finnish	.2
Spanish	8.	Scandinavian	1.1	Turkish	.2
German	7.4	Hungarian	.8	Other	.6
Slavic	7.2				

All entries for articles from these journals will be supplied in the vernacular, with the title translated, in addition, into English.

Relation to QCIM. Adding the titles indexed by *QCIM* to those indexed by the *Current List* gives a total of almost 2,000. One-third of this total is covered by both indexes. Approximately one-third of the total is therefore covered uniquely by each index.

There is sound justification for this overlap. For years coordination of these two publications has been discussed. The Library wishes to demonstrate that it is possible to publish in a monthly form material similar to that published by *QCIM*. On the basis of its experience, and on the common ground established by common indexing practices, it should be possible at some date in the not too distant future to arrive at agreements leading to close collaboration, or even to the establishment of a single comprehensive medical index.

Convenience. It is generally maintained that the most convenient form for a medical index is the dictionary arrangement established by the *QCIM*. Considerations of speed and economy required that the Library compromise with this ideal form. Repetition of citations adds to labor costs, takes printing space, and delays publication. Consequently the following mechanical features, which offer a maximum speed with acceptable convenience, have been adopted.

The *Current List* will be published each month in two sections within a single cover: a register and an index. Arrangement of the register section will be alphabetically by title of the journal issue indexed. The caption will carry necessary bibliographic information and an abbreviation. Under each caption will be entered, with inclusive page references, the articles in the order in which they appear.

An identifying serial number will be assigned to each article, starting with "1" for the first article of the first issue of a year, and running to approximately 80,000 for the last article in the last issue. Thus the register sections for each month may be added together, establishing a consecutively numbered series of references for the year.

The second section in each monthly issue will be a combined author and subject index, whose entries will refer to the numbered articles in the register section. These monthly indexes may be used individually throughout the year. They will be

replaced at the close of the volume with a cumulated author and subject index, which can then be bound with the combined register sections.

Format. To insure a maximum utilization of space, the page size will be increased to 9⅛ by 11⅜ inches. The register section will have three columns, and the index section six. Each monthly issue will contain between 200 and 300 pages.

Techniques. Throughout the planning of this publication new techniques have been sought and adopted. The copy will be typed by proportionate-spacing electromatic book-face typewriters on strips of perforated cards. One strip, representing main entry, authors, and assigned subjects, each on an individual card, will be made for each article. The strips will be filed by journal title until the end of the month. To assemble the *Current List*, all cards for each article will be given an identifying serial number. The cards will then be separated. All main entries will be mounted in consecutive order under the name of the journal, and will be photographed for offset printing as the register.

Cards representing subject and author entries will be interfiled alphabetically, then mounted and photographed for offset printing as the index section; these same index cards will be refiled and interfiled with the cards for the indexes of succeeding months, so that at the end of the year a cumulative author and subject index can be published, using the original copy for the purpose.

Thus the *Current List* will be prompt to publish, will cover regularly the literature of importance to contemporary medical research, will be expanded to cover more in the future, and will be provided with a cumulative feature. Its form is frankly experimental, and modifications may be expected. It does, however, provide solutions for some of the problems which have plagued makers and users of indexes.

The Army Medical Library Author Catalog

The *Current List of Medical Literature* will not include the monographic literature received and catalogued in the Library. This will be the function of an annually published *Army Medical Library Author Catalog*.

The Army Medical Library has recently established a new co-operative cataloging agreement with the Library of Congress. Under this new plan, the Army Medical Library will prepare catalog card copy for all titles which lack Library of Congress catalog cards. This copy will be printed by the Library of Congress, where sets of cards will be distributed by the Card Division at a price of $100 per year. Approximately 10,000 cards will be supplied on each subscription, bringing the net price per title down to one cent.

In continuation of a program initiated with the publication of *Army Medical Library Catalog Cards* (April-December 1948), the Library of Congress will assemble and print a set of these cards as *The Army Medical Library Author Catalog*. This annual publication will be in a format similar to, and will supplement, the *Library of Congress Author Catalog*. The volume covering the Library's 1949 cataloging contains approximately 23,000 main and added entries for monographic and serial titles.*

The Army Medical Library Author Catalog is planned as an annual publication. It has an important place in the new bibliographical program established by the Library, in that it supplements the *Current List* and replaces the publication of entries for monographs and serial titles in the *Index-Catalogue*. It will provide librarians with authority for name forms, and bibliographers with an annual record containing among other things the titles of newly published medical works received in the Library during the preceding year. In that it contains entries for volumes processed in the comprehensive recataloging program, the successive volumes will provide a printed author catalog for the Army Medical Library.

There remains the problem of capitalizing on the long term investment represented by the 1,750,000 unprinted references in the *Index-Catalogue* Subject File. On this question the Committee made two recommendations (their recommendations 3 and 4 as listed previously on page 284). There are approximately 420,000 unpublished cards referring to monographic literature by author, by subject, and by author as subject. These

*Copies of this printed catalog may be purchased from the Card Division, Library of Congress, Washington 25, D. C., for $12.50.

entries will presumably make a five-volume supplement of approximately 1,400 pages each. However, in view of the extensive editorial preparation required (cross references, reassignment of subjects, etc.) the manuscript for all five volumes will have to be completed before the first volume can be published. Staff has already been asssigned to this project but it will inevitably take a number of years before the editing and publication of this supplement are realized. The supplement will contain monographs received up to 1950; those received in succeeding years will be published in *The Army Medical Library Author Catalog.*

After 61,000 cards for the 11th volume, Fourth Series, *Index-Catalogue,* and 75,000 subject cards for the supplement of monographs have been removed for publication, there will remain approximately 1,530,000 cards representing articles in serial literature arranged from A to Z under approximately 40,000 *subjects.*

Since many of these cards were rejected for publication in the Second and Third Series, they represent indexing done from 1900 to date of publications of three centuries. They are in many styles, and in many cases under obsolete or tentative subject headings.

Advantageous utilization of these cards is a major problem. Physicians and librarians alike believe that there are thousands of references which are inconsequential, superseded, or otherwise without value. The Library is under a mandate to preserve through publication the values inherent in this file.

Several possibilities are apparent:

1. The references might be selected according to the scientific value of each article. This is a tedious process, involving scanning the original paper, and requiring a large staff of highly qualified employees.
2. The references might be selected by date, publishing references dated 1940–50. The Library's date-filing would facilitate this selection.
3. All the cards might be coded and filmed for high speed scanning and selection by the Shaw-Bush Rapid Selector. This machine views microfilm at the rate of 100,000

frames per minute, selects desired references, photographs them, and provides the inquirer with a bibliography on microfilm. This would involve re-examination of all the material and the labor of coding, which would be enormous.

4. The cards might be used as a basis for extensive bibliographical publications in such fields as cancer, tuberculosis, heart disease, or any other in which a great current need exists. The preparation and publication of such bibliographies might be undertaken by the Library alone, or in conjunction with another Federal or private agency.

Programs for utilizing these references are under study by the Library and by the Indexing Committee. They represent a tremendous investment of funds, personnel and effort, and they must produce results which will have commensurate social values.

Summary

It is fitting in this mid-century year to look at the past and to anticipate the future. Medical bibliography is at the divide.

The *Index-Catalogue*, planned by Billings in more leisurely days, has survived the first half of the twentieth century in a state of continuous crisis. Organized and subsidized research has resulted in an explosive acceleration of the literature of medicine, and the alphabetical series of the *Index-Catalogue* has not been able to cope with the problem. Every year the unpublished files increase; by 1957 more references would have been withheld from publication than have been published. A committee of experts, speaking with the authority of both the medical and library professions, after two years of study, has recommended its discontinuance and the substitution of a new publication program.

In developing this program, the Army Medical Library has attempted to provide for the indexing of the significant current literature of the medical sciences by reconstructing the *Current List of Medical Literature*. The recording of new monographic literature will be taken care of by the new co-operative cataloging agreement with the Library of Congress, resulting in the annual publication of the *Army Medical Library Author Catalog*.

The Library will publish one more volume of the *Index-Catalogue* (Vol. XI, MH-MZ), and will publish a five-volume supplement to the *Index-Catalogue* to include subject entries for monographs now in file. Selections will be made from the 1,530,000 references by subject to journal literature remaining, and a publication program capitalizing on the investment made throughout the years will be launched.

Medical bibliography is a matter of concern to the entire medical profession and to the scientific world. It must both reflect and guide the purposes, the methods, and the results of scientific inquiry. It must, therefore, be integrated with research programs as they are foreseen, even though dimly, and as they are comprehended, even though imperfectly, by the makers of bibliographies and indexes. Medicine today can ill afford the luxury of encyclopedic bibliography purely for bibliography's sake. It can afford, and should support with all possible vigor, any bibliographical activity which will aid in scientific communication, advance the frontiers of knowledge, and benefit the society from which it springs and to which it owes its existence.

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CALLING ATTENTION TO:

Items of Possible Interest to Our Friends

March, 1950

Cancerous: CH Andrews reviews virus theory of cancer (*Brit Med J* 1/81/Jan 14/50). JS Fruton notes action of proteolytic enzymes in synthesis of peptids (*Yale J Biol Med* 22/263/50). L Kiefer & &Co (*Proc Soc Exp Biol Med* 73/29/50) confirm CB Huggins & Co (*Concer Res* 9/117/49) on average values on healthy persons of iodoacetate index in serum coagulation test for cancer. VR Potter & Co show multiplication of mitachondria is independent of cell multiplication & propose enzyme deletion hypothesis of carcinogenesis (*Cancer Res* 10/28/50). R Schrek & Co find significant correlation between cigarette smoking & resp tract cancer (*Cancer Res* 10/49/50).

Iatrous: MD Altschule analyses *Physiology in Diseases of Heart & Lung* (Harvard Press, Cambridge, 49, 368pp, $5). MF Boyd edits comprehensive *Malariology* (Saunders, Phila, 49, 2 vols, 1643pp, $35). G Brownlee offers experimental evidence for use of deoxycorton acetate & ascorbic acid in arthritis (*Lancet* 1/157/Jan 28/50). JH Cort & GD Davis note influence of vagus in symptoms of pulmonary embolism (*Yale J Biol Med* 22/213/50). J Delay notes thalamic degeneration after prefrontal lobotomy (*Presse Med* 58/77/Jan/28/50). GL Engel discusses *Fainting: Mechanisms & Diagnosis* (CC Thomas, Springfield, Ill, 50, 115pp $2.5). BS Hetzel appraises liver function tests (*Med J Austral* 1/1/Jan 7/50). G. W. Hofman & Co discuss lipids in atherosclerosis (*Science* 111/166/Feb 17/50). C Heymans writes *Introduction to Regulation of Blood Pressure & Heart Rate* (CC Thomas, Springfield, Ill, 50, 66pp, $1.75). SP Hicks studies brain metabolism *in vivo*, showing selective destruction by cyanide of white matter, which is low in cytochrome oxidase (*Arch Path* 49/111/50). R Kovacs describes *Light Therapy* (CC Thomas, Springfield, Ill, 50, 117pp, $2). D LeVay & GE Loxton show peripheral action of deoxycorton acetate & Ascorbic acid in relieving arthritis (*Lancet* 1/209/Feb 4/50). HL Marriott discusses *Water & Salt Depletion* (CC Thomas, Springfield, Ill, 50, 88pp, $2). FW McKee & Co describe circulation of ascitic fluid (*J Exp Med* 91/115/50). N Marko: discusses chronic epidemic hepatitis (*Schw Med Wchnschr* 80/93/Feb 4/50). J Marmor & Co classify mental disorder in relation to individual stability & environmental stress (*J Nerv Ment Dis* 111/19/50). LM Morrison & WF Gonzales show choline reduces mortality rate from thrombosis in proven coronary atherosclerosis (*Proc Soc Exp Biol Med* 73/37/50). National Health Assembly reports on *America's Health* (Harper's NY, 49, 395pp, $4.5). H Persky & Co show hypersecretion of hippuric acid after benzoate in anxiety states (*J Clin Invest* 29/110/50). HH Schrenk discusses smog (*Arch Indust Hyg Occup Med* 1/189/50). GEM Scott analyses the coeliac syndrome (*Med J*

Austral 1/37/Jan 14/50). IF Commerville & Co show high pregnanediol excretion after progesterone in rheumatoid arthritis (*Lancet* 1/116/Jan 21/50). E Weiss offers *Principles of Psychodynamics* (Grune & Stratton, NY, 50, 268pp, $4). WF Windle discusses *Asphyxia Neonatorum* (CC Thomas, Springfield, Ill, 50, 67pp, $2).

PHARMACOLOGOUS: HK Beecher & Co find no significant acidosis in clinical ether anesthesia (*J Pharmacol* 98/38/50). P Hofmann discusses pathogenesis & treatment of vasomotor head-ache with dihydroergotamine (*Schw Med Wchnschr* 80/28/Jan 14/50). CA Lawrence discusses *Surface-Active Quarternary Ammonium Germicides* (Academic Press, NY, 49, 240pp, $6). MM Nelson & Co show decreased bone & blood formation in pantothenic acid deficiency (*Proc Soc Exp Biol Med* 73/31/50). RM Nelson & Co note need of potassium in dehydrated surgical patient (*Surg* 27/26/50). E Rost analyses metabolism of straw bread (*Ztschr Immu Exp Therap* 107/7/50). FH Shaw & G Bentley show acridine antagonism to morphine (*Med J Austral* 2/868/Dec 17/49). H Tauber describes *Chemistry & Technology of Enzymes* (Wiley, NY, 49, 550pp, $7.5). S Wolf discusses pharmacological effects of placebos (*J Clin Invest* 29/100/50).

CULTUROUS: TR Forbes discusses history of witch's milk & marks (*Yale J Biol Med* 22/219/50). BL Gordon surveys *Medicine Throughout Antiquity* (Davis, Phila, 49, 828pp, $6). M Pei writes *The Story of Language* (Lippincott, Phila, 49, 495pp, $4). J Romano edits *Adaptation* (Cornell Press, Ithaca, 49, 113pp, $2). JBdeCM Saunders & CD O'Malley discuss *The Illustrations from the Works of Andreas Vesalius* (World Publ, NY, 50, 276pp, $10). GG Simpson in Terry Lectures on *The Meaning of Evolution* (Yale Press, New Haven, 49, 389pp, $3.75) supports ethicogenesis (*Sci Month* 60/245/45). EH Thomson describes *Harvey Cushing: Surgeon, Author, Artist* (Schuman, NY, 50, 280pp, $3.5):

APRIL, 1950

CULTURAL: C Bernard's great *Introduction to the Study of Experimental Medicine* is again available (Schuman, NY, '50, 200pp, $3)—1st published in Paris in 1865, MC Greene translated the volume in 1927 (Macmillan, NY, '27, 246pp), & it promptly went out of print. E. W. Count compiles *This Is Race: An Anthology Selected from the International Literature on the Races of Man* (Schuman, NY, '50, 747pp, $7.5). J Dewey & AF Bentley discuss *Knowing and the Known* (Beacon, Boston, '49, 334pp, $4). A Einstein offers 3rd ed. of *The Meaning of Relativity*, with 1st publication of "The Generalized Theory of Gravitation" (Princeton Univ. Press, '50, 150pp, $2.5). L Evans discusses present state & possibilities of improving *Bibliographical Services* (Unesco-Library of Congress Bibliographical Survey, Washington, '50, 67pp, to which KO Murra adds an appendix on the development of current complete national bibliographies). J Hirsh ably discusses *The Problem Drinker* (Duell, Sloane & Pearce, NY, '49, 223pp, $3). M Johnson offers *Art & Scientific Thought: Historical*

Studies toward a Modern Revision of Their Antagonism (Columbia Univ. NY, '49, 200pp, $3). M Otto collects essays on *Science & the Moral Life* (Mentor Books, NY, '49, 192pp, $0.35). JH Powell recalls *Bring Out Your Dead: The Great Plague of Yellow Fever in Philadelphia in 1793* (Univ. Pennsylvania, Phila, '49, 314pp, $3.75). MC Rathbun & BH Hayes, Jr, prepare admirable *Layman's Guide to Modern Art in a Scientific Age* (Oxford Press, NY, '50, no pagination, $4). A Standen is smartly annoying in *Science Is a Sacred Cow* (Dutton, NY, 221pp, '50, $2.75). R West discusses the origins of war in the minds of men (*Lancet* 1:414/Mch 4/50). C Zirkle deplores *Death of a Science in Russia: the Fate of Genetics as Described in Pravda* (Univ Pennsylvania Press, Phila, '49, 333pp, $3.75). There is evidence of decay of medicine in Soviet Russia, due to purge of "bourgeois theories (Virchow, Pasteur, Koch & Ehrlich) of pathology" (NY Times, Feb 28/50, p 19).

CLINICAL: W Antopol finds high doses of cortisone may produce exhaustion of protective mechanisms (*Proc Soc Exp Biol Med* 73/262/50). KL Armstrong & Co show specific influence of vitamin B_6 on D-amino acid metabolism (*ibid* p 159). TG Armstrong & Co find penicillin & sulfasuxidine useful in amebic dysentery by destroying organisms symbiotic with amebae (*S Afr Med J* 24/121/Feb 25/50). JRG Bradfield discusses localization of enzymes in cells (*Biol Rev Cambr Phil Soc* 25/113/50). AJ Cain analyses histochemistry of lipoids in animals (*ibid* p 73). PN Campbell & Co isolate a crystalline toxic factor, probably of methionine derivation, from agenized wheat flour (*Nature* 165/345/Mch 4/50). CB Chapman & TB Gibbons review diet & hypertension (*Medicine* 29/29/50). M Chieffi show skin massage with estrogen in oil improves elastic properties of old female skin, but androgens have no effect on male skins (*J Gerontol* 5/17/50). HI Chinn & FW Oberst find hyoscine (0.65 mgm) & benadryl (50 mgm) more effective in controlling air-sickness than other drugs (*Proc Soc Exp Biol Med* 73/218/50). M Dubuisson discusses muscle activity & muscle proteins (*Biol Rev Cambr Phil Soc* 25/46/50). D Hamre & Co show aminobenaldegyde-thiocarbazone is effective in vaccinia virus (*Proc Soc Exp Biol Med* 73/275/50). E Jacobson & V Larsen discuss formation of acetaldehyde after antabuse & alcohol (*Acta Pharmacol* 5/285, 292/49). PH Jordan & Co show hyperglycemia causes eosinopenia (*Proc Soc Exp Biol Med* 73/243/50). SA Komarov & Co find Na dodecyl sulfate selectively stimulates mucous secretory cells of stomach (*Brit J Pharmacol* 5/1/50). A Linksz offers *Physiology of the Eye: Optics* (Grune & Stratton, NY, 50, 334pp, $7.5). AG Macgregor & DR Wood report Na salicylate protects against myocarditis (*Brit J Pharmacol* 5/9/50). D Nachmansohn edits *Metabolism & Function: A Collection of Papers Dedicated to Otto Myerhof on His 65th Birthday* (Elsevir, NY, 50, 348pp, $7). A Piney thinks healthy blood contains an antileukemic factor (*Acta Haematol* 3/3/50). DE Smith & Co find cysteine protects against X-radiation (*Proc Soc Exp Biol Med* 73/198/50). K Van Dongen & A Taal discuss mechanism of cardiac fibrillation (*Arch Internat Pharmaco* 81/129/50).

HL Zauder & NB Dreyer find adenine depressor substance in essential hypertension (*ibid* p 245).

MAY, 1950

CLINICAL: FK Albrecht analyses problem of obscure fever (over-slick *GP* 1/33/50). AR Arias & Co report histidine & ascorbic acid help in chronic obliterating arterial disease (*Angiol* 2/1/50). DB Cheek & CB Hicks describe pink disease or infant acrodynia (*Med J Austral* 1/101, 107/Jan 28/50). HR Cooper discusses forward and backward heart failure hypotheses (*USAF Med J* 1/377/50). EH Cranswick & TC Hall find deoxycortone & ascorbic acid useful in mental disorder (*Lancet* 1/540/Mch 25/50). R Eker notes protein deficient diets induce kidney changes like "lower nephronnephrosis" (*Scand J Lab Clin Invest* 2/62/50). JC Freijo reviews histoplasmosis (*An Fac Med Montevideo* 34/1049/49). JR Gallagher outlines diagnosis of specific language disability (*New Eng J Med* 242/436/Mch 23/50). E Grandjean & Co (independent reports) claim water-soluble protein-free heart-muscle extract ("recosin") improves cardiac function in myocardial degeneration (*Schw Med Wchnschr* 80/203–210/Feb 25/50). H Haubold discusses carotinoids in thyroid equilibrium (*Arzt Forsch* 4/149/Mch 25/50). SP Hicks & S Warren offer slick *Introduction to Neuropathology* (McGraw-Hill, NY, 50, 500pp. $10). N Jolliffe, FF Tisdall & PR Cannon edit *Clinical Nutrition* (Hoeber, NY, 50, 925pp, $12). A Keys & Co edit *Biology of Human Starvation* (Univ Minnesota Press, Minneapolis, 50, 2 vols, 1360pp, $19.5). F Kunchel & Co sympose on thiosemicarbazone in TB (*Artz Forsch* 4/88–104/Feb 25/50). K Langner confirms specific antigenic reaction with leucocytes in TB (*Klin Wchnschr* 28/177/Mch 15/50). I Maldonado-Allende & PB Componova discuss salicylate toxicosis (*Prensa Med Argent* 37/473/Mch 10/50). J Mallarme & L Heilmeyer analyse hemolytic anemia (*Sang* 21/89, 105/50). M Morley & Co discuss childhood speech disorders & the family doctor (*Brit Med J* 1/574/Mch 11/50). R Pulver & Co report on value in rheumatism of intramuscular injection of "irgapyrin," new pyrazolidin derivative (*Schw Med Wchnschr* 80/308, 310/Mch 25/50). Note Scandinavian Symposium on kidney in health & disease (*Scand J Lab Clin Invest* 1/263–353/49). LA Watson & TL Tolan review *Hearing Tests & Hearing Instruments* (Williams & Wilkins Balt, 50, 607pp, $7). BB Wells writes *Clinical Pathology* for practitioners (Saunders, Phila, 50, 397pp. $6). RW Wilkins appraises veratrum viride in hypertension (*New Eng J Med* 242/535/Apr 6/50).

BIOLOGICAL: KH Beyer discusses *Pharmacological Basis of Penicillin Therapy* (CC Thomas, Springfield, Ill, 50, 170pp, $4). J. Campbell & IRH Kramer note streptomycin relaxes gut (*Nature* 165/78/Jan 14/50). ERA Cooper discusses development of thalamus (*Acta Anat* 9/201/50). D Eckert & G Totterman show evidence against histamine being essential part of eosinophil granules (*Scand J Lab Clin Invest* 2/58/50). L Farmer & GL Rohdenberg demonstrate histamine induced cardiovascular lesions

(*J. Allergy* 21/120/50). German Pharmacology Society 1948 meeting results in 80 abstracts (*Arch Exp Path Pharmakol* 208/1–228/49). Federation Proc contains about 3000 abstracts from nearly as many authors (*Fed Proc* 9/1–399/50). M Herland & PS Timiras find increase in alkaline phosphatase action in all organs except kidneys in animals exposed to stress (*Endocrin* 46/243/50). NO Kjeldgaard finds antabuse inhibits liver aldehyde oxidase (*Acta Pharmacol Tox* 5/397/49). AB Lerner & TB Fitzpatrick survey melanin formation (*Physiol Rev* 30/91/50). CE Lumsden shows constant symmetrical lesion of cerebral white matter from chronic KCN poisoning, & supports O Marburg (*Jb Psychiat* 27/213/06) in saying demyelinating diseases are caused by myelinolytic ferment liberated in brain (*J Neurol Neurosurg Psych* 13/1/50). JM Rogoff & Co say epinephrine secretion by adrenals diminishes blood potassium (*Proc Soc Exp Biol Med* 73/163/50). CF Schmidt discusses *The Cerebral Circulation in Health & Disease* (CC Thomas, Springfield, Ill, 50, 88pp, $2). KF Wells offers *Kinesiology: The Mechanical & Anatomic Fundamentals of Human Motion* (Saunders, Phila, 50, 478pp, $4.75).

GENERAL: JS Collings surveys general practice in England today (*Lancet* 1/555/Mch 25/50). FK Bolan documents *The First Anesthetic: The Story of Crawford Long* (Univ Georgia Press, Athens, 50, 160pp, $3). WAR Leys relates *Ethics and Social Policy* (Prentice-Hall, NY, 50, 522pp, $5). G Gask collects his essays on *The History of Medicine* (Butterworth, London, 50, 420pp, 30s). Sir Heneage Ogilvie warns us of the "granny racket" in shrewdly appraising the British state medicine system (*Brit Med* J 1/683/Mch 25/50). R Payne probes the doctrine of death (*Sat Rev Lit* Apr 15/15, p11). A Rapoport offers *Science & the Goals of Man: A Study in Semantic Orientation* (Harper's NY, 50, 280pp, $3.5).